HEALTH EFFECTS OF HYDROGEN SULPHIDE:

Knowledge Gaps

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FOREWORD

As a result of a multi-stakeholder workshop in 1996, the Alberta Council for Research on Air and Health (ACRAH) was formed "to develop a more efficient strategy for linking the needs and resources for research in human health and air quality in Alberta." The Council is composed of representatives from industry, government, health organizations, environmental organizations, universities and research institutions. It identifies, prioritizes, promotes and facilitates research about air quality and health. It coordinates a multi-disciplinary, province-wide network of scientists, investigators, and technical experts. The Council is a coordinating and advisory body without funds of its own to fund research.

The Board of the Clean Air Strategic Alliance (CASA) approved a recommendation from the CASA Air Toxics Project Team on June 24, 1998 that *"a research program be developed and funded to address the knowledge gaps related to the health effects database for* H_2S*"*, and asked ACRAH to *"identify priorities and determine who could do the research."*

ACRAH presented a four-part proposal to answer outstanding questions about the health effects of H_2S at the June 17, 1999 CASA Board Meeting, which the Board approved in principle. It included a search of background information about what is known about H_2S.

In response to the proposal submitted in 1999 by the Alberta Lung Association, Alberta Environment approved funding for the search of background information on hydrogen sulphide.

Drafts of this report have been reviewed by outside experts and revisions have been made on successive copies. The report is not a scientific document but describes a broad range of scientific literature and local observations. Although it is not a critical scientific review, it does identify knowledge gaps about the health effects of hydrogen sulphide and may be useful in designing future research projects.

Dennis Stokes, Ph.D.
Project Coordinator

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SUMMARY

Exposure to H_2S and its consequent health effects continues to be of great concern to many Albertans. A mandate from the Clean Air Strategic Alliance (CASA) in June 1998 stated that the health effects of H_2S was a priority, and that the Alberta Council for Research on Air and Health (ACRAH) was to develop a research plan for H_2S research. One of the directives to ACRAH was to identify knowledge gaps for H_2S toxicity.

This report addresses knowledge gaps regarding the health effects of H_2S. Although this report is focused on H_2S, it was regarded necessary to include some references to "mixtures" such as sour gas, sewer and landfill gas, and pulp and paper emissions since these discharges contain H_2S. Reports including scientific and medical literature regarding health effects of H_2S published between 1990 and 2001, hereafter referred to as 'the past decade', were located. The studies are organized alphabetically by organ system affected, listed chronologically with the most recent first and summarized in tables for brevity and clarity. The studies are also broadly grouped into human, animal and *in vitro* studies. In addition to the scientific literature, other sources of information including media reports, documented personal accounts, reports from other institutions, medical, agricultural, veterinary facilities, research boards, and workers' compensation boards were examined if available.

A few studies suggested, but not confirm, that H_2S has carcinogenic potential. The majority did not control for co-exposures or exposure measures which severely limit the detection of causal relationships. The nasal cavity appears a target site for increased cancer risk. Additional studies are required to clearly define the cause/effect relationship, and long-term studies in animal species and supplementary *in vitro* studies are warranted designed to assess the carcinogenic potential of H_2S per se, as well as in combination with other substances.

Studies of workers have shown slight increased risks for cardiovascular disease. Few controlled for other known cardiovascular risk factors or collected good exposure data. It is difficult to assess what proportion of risk is related to H_2S exposure. Recently, it has been reported that endogenous H_2S functions as a neuromodulator suggesting a key role in vascular tone, endothelial cell function and blood pressure, thus the effects of exogenous H_2S on vascular tone should be examined in more detail.

A few studies explored the relationship between H_2S and the endocrine system, growth and reproduction, including effects on carbohydrate metabolism, smooth muscle function and core temperature control. Research addressing effects on hormone cascades, thyroid function, diabetes, growth *in utero* and postnatal is still lacking.

Recent studies have further illustrated, although not completely, the relationship between low level exposure and effects on the gastrointestinal system supporting earlier work. Since subjects were also exposed to other substances it is difficult to ascribe the effects of symptoms to H_2S per se. Further clarification of time-concentration relationships of low level exposures and determination of threshold levels would be valuable. In addition, the role of the olfactory system in triggering the gastrointestinal responses should be examined.

Although animal studies suggest reduced weight gains associated with H_2S exposure, human studies evaluating the effects of H_2S exposure on growth and development were not conducted. Controlled experimental studies are required.

Further studies examining the effects of H_2S on specific hematology parameters are required.

A few studies reported a relationship between hepatic function and H_2S exposure but provided a minimum of exposure-response data. Studies should be undertaken to evaluate whether there is a combined effect of H_2S and mercaptans since there is suggestive evidence that low concentrations of methyl mercaptan may affect liver function.

Several reports noted alterations in immune function, however many of these were *in vitro* studies. Additional research on both humoral and cellular immunity are required to establish dose-response relationships.

Although many reports described the effects of H_2S (and methyl mercaptan) on various tissues such as dermal, periodontal, gastrointestinal, and ocular mucosa, additional studies should be undertaken to assess eye symptom prevalence, visual acuity, and ocular diseases with simultaneous measurement of concentrations.

In studies of human exposure, a variety of effects on the nervous system have been reported. Alterations in neurophysiological and neuropsychological tests and increased frequencies of symptoms have been found following both short-term high-level exposures and long-term low-level exposures. Few of the human studies obtained good exposure data that could be clearly associated with the reported effects. Many diverse neurological effects are reported across a wide concentration range. Thus it still remains controversial whether very low levels result in neurological effects. Additional studies should also be undertaken to assess the effects on brain chemistry, physiology and function.

Recent literature suggests that odours may have more than aesthetic qualities, and may be a contributing factor in determining health. Odours, especially aversive odours, have multiple impacts on humans, producing changes in emotion, physiology, behavior, and other responses, thus greater attention should be given to studying these interactions.

Only one study was located that specifically addressed the relationship between H_2S and renal function, however this was not regarded as a high priority.

Many anecdotal reports drew attention to concerns about an association between sour gas exposures (i.e. H_2S) and altered reproductive outcomes, however, few controlled studies were undertaken that examined the relationship between reproduction and H_2S exposure. There is considerable discrepancy between the reproductive effects reported in animal experiments and those given in the anecdotal reports. Further research must be conducted both *in vivo* and *in vitro* using a variety of systems to clarify the reproductive effects of H_2S alone and in combination with other gases and vapors.

Many studies provided a better understanding of respiratory health effects following acute and long-term low level H_2S exposures. Additional studies are required that address interactions and long term effects of mixtures.

Few studies examined H_2S toxicokinetics (i.e absorption, distribution, metabolism or elimination). Although there is some limited information on the distribution and metabolism, there were no studies that examined absorption or elimination.

There are many examples that H_2S should be regarded as a broad-spectrum toxicant, and that repeated exposure may result in cumulative effects[1] on many organ systems such as the brain, lung and heart. In addition, there is evidence that cumulative health effects of repeated low-level H_2S exposure exist, which does not support earlier claims that H_2S is only an acute toxicant due to its rapid metabolism to non-toxic products. It still remains a challenge to conclude what levels of exposure to H_2S pose a health risk to the general population and the sensitive individual. An understanding of exposure-response relationships is of primary importance and priority should be given to establish dose-response curves in all areas of investigation.

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[1] Cumulative effects means the combined and accumulated biological impacts of repeated hydrogen sulphide exposure on cells, organs and animals or animal populations which may lead to pathological changes or disease outright.

1.0 INTRODUCTION

Exposure to H_2S and its consequent health effects continues to be of great concern to many Albertans. Albertans are also apprehensive about the combined effects of H_2S with other sulphur compounds such as mercaptans and other pollutants in general. A Mandate from CASA (Clean Air Strategic Alliance) in June 1998 stated that the health effects of H_2S were a priority, and that ACRAH (Alberta Council for Research on Air and Health) was to develop a research plan for H_2S research. One of the directives to ACRAH was to identify knowledge gaps for H_2S toxicity. This report addresses knowledge gaps regarding the health effects of H_2S. Hydrogen sulphide usually exists in mixtures such as sour gas and often with other pollutants. In a few cases, it has been studied as a 'mixture'. Therefore some of these studies were included in this report.

Reports published between 1990 and 2001, hereafter referred to as 'the past decade', were reviewed. The studies are organized alphabetically by organ system affected, and are listed chronologically with the most recent first. The data have been summarized in tables for brevity and clarity. The studies are also broadly grouped into human, animal and *in vitro* studies.

Several recent reviews of the H_2S literature are available (Agency for Toxic Substances and Disease Registry, 2000; US EPA, 1993; Schiffman et al., 2000; Michigan Environmental Science Board Hydrogen Sulfide Investigation Panel, 2000; Petroleum Communication Foundation, 2000a, 2000b; the complete issue of Environmental Epidemiology and Toxicology, 1999: Vol. 1, No. 3-4). A symposium held in October 1999 at Chapel Hill, North Carolina, discussed current H_2S research. The proceedings can be found at the website <http://www.api.org/ehs/h2s/>. A wide variety of symptoms have been described following exposure to H_2S. While many organ systems have been affected by H_2S, the olfactory, respiratory and nervous systems appear to be most affected at low concentrations (Reiffenstein et al, 1992; ATSDR, 1999). The effects of H_2S on most organ systems suggest that it is a broad-spectrum toxicant.

In the majority of studies that were reviewed, concentration measurements of H_2S were not obtained at the time and location of the exposures. Published workplace studies have rarely provided good exposure measurements even though a wide variety of monitoring and sampling methods have been available for decades[2]. With this consistent problem, it should be noted that for any indoor or outdoor emission source, the airborne concentrations at any time period in the vicinity of that source could vary by several to many orders of magnitude within short time periods (Yee et al., 1993; Hilderman et al., 1999). Measurements at a sewage purification plant where continuous measurements over several 3-week periods showed that the concentrations generally were lower than 2 ppm, but peak concentrations over 100 ppm were discovered (Sostrand et al., 2000). Rapid onset and decline characterized these peak concentrations that occurred at regular intervals. Short-term concentrations or instantaneous concentrations in the outdoors will have the largest distribution of values (Battelle Pacific Northwest Laboratories, 1979). Therefore the signs and symptoms reported cannot easily be associated directly with a given concentration or even a limited concentration range.

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[2] The American Industrial Hygiene Association Journal publishes annual buyers guides for a wide variety of instrumentation, including monitors for H_2S.

This situation is further complicated by a steep dose-response curve for H_2S (Prior et al., 1988) where the concentration range between "physiological and toxicological" concentrations of sulphide in tissues is only within a 2-3-fold difference (Warenycia et al., 1989b). Thus, with the exception of controlled laboratory exposures, it is difficult to accurately identify exposure concentrations for most health endpoints.

Scientific and medical references were obtained by searching the following databases using the keywords 'hydrogen sulphide', 'sour gas' or 'reduced sulphur': Embase, Toxline, Medline, Biosys, Biological Abstracts, Biological Sciences, Biological Digest, Biological and Agricultural Index, RussianMedline (DIMDI), RTECS, NIOSHTIC, IRIS, HSDB, AGRICOLA, CancerLit, CCRIS, CCOHS-CHEMINFO, Conference Papers Index, Current Contents, DART/ETIC, Environment Abstracts, NTIS, Health and Safety Science Abstracts, and Zoological Record.

Government websites were also searched including EPA scientific reports, ATSDR Toxicity Profiles and NIOSH Criteria Documents. Media and documented anecdotal reports were obtained by searching the Canadian Newswire, Canadian NewsDisc, and the Internet using the following search engines: Google, Yahoo, Hotbot, Lycos. Also, information inquiries were made of relevant organizations listed in the Alberta Environmental Network Directory. Attempts to obtain anecdotal H_2S reports, in a readily accessible format, from the Alberta Worker's Compensation Board, H_2S training groups, Alberta Labor, Physician or Veterinarian Associations, were of limited success.

The research into the health effects of H_2S during the decade has revealed that the biological effects of H_2S are diverse and complex. A greater understanding of the possible roles of H_2S in the nervous system as a developmental toxicant; in the endocrine system as a neuro-modulator; in the lung as a respiratory center regulator; and as an odour-induced general stressor has been demonstrated in a number of reports. These effects may be intricately linked to one another, thus it is difficult to fully understand their overall biological impacts.

During the past decade, the realization that there is a complicated relationship between H_2S and the nervous system emerged. It has been suggested that H_2S acts as a neuromodulator at low levels and produces a wide variety of toxic effects at levels only one or two orders of magnitude higher. Some neurophysiological effects following exposure to concentrations of H_2S in the range of 10 - 100 ppm include dizziness, fatigue, headache, lethargy, loss of appetite, abnormal peripheral reflexes, mental depression, irritability, poor memory (Alberta Health, 1988; Reiffenstein et al, 1992; ATSDR, 1999; Roth, 1999).

Significant advances during the past decade have also been made in understanding the effects of H_2S on the cardiovascular system. Prior to 1990, many studies suggested that the heart was sensitive to the effects of H_2S (Table 2). In the past decade, several large epidemiological studies have repeatedly demonstrated a slight but increased risk for cardiovascular disease in H_2S exposed groups (Table 2). Also, it has been shown that H_2S poisoning by inhalation distributes the toxicant primarily to the heart and lung (Ikebuchi et al., 1993). With some exceptions, animal and biochemical studies have also provided evidence in support of this relationship (Table 2).

Furthermore, the effects of odours on the cardiovascular system, which have recently been reviewed by Smith and colleagues (1999), should be noted. Although the authors found that the literature was incomplete, odours appeared to induce neuro-endocrine or reflex changes in the cardiovascular system, i.e. stress responses. Adverse (sensory) reactions to strong odours and irritants may lead to neuro-endocrine changes such as the release of the stress hormones, catecholamines and cortisol, which then lead to increased heart rate, elevated blood pressure, vasoconstriction, platelet aggregation and alterations in the blood lipid profile (high-density lipoprotein, cholesterol, and triglycerides). These are common cardiovascular risk factors. In addition, chronic stress or cortisol excess sustained over long periods may have damaging effects on the brain (Sapolsky, 1996). These findings are especially significant since negative mental health (stress, anxiety, frustration, worry) associated with living or working near sour gas facilities were among participants' top concerns presented at open house meetings of the Alberta Public Safety and Sour Gas Provincial Advisory Committee (Appendix II). Participants in the CASA Animal Health Workshop (1999; p 71) described their experiences: being scared, learning to live with stress and anxiety of not knowing what the day will bring, and difficulty getting assistance from regulatory agencies and the oil or gas company alike. Compared to reference groups, worry about general health was also found more often in people living near a natural gas refinery (Spitzer et al., 1989), and compared to a reference period of low emissions, increased depression and anxiety were reported in a community living downwind of a paper mill that released a strong malodorous emission containing H_2S during introduction of new processing method (Haahtela et al., 1992).

Significant gains during the past decade have also been made in understanding the cellular effects of H_2S from studies of various mucosal tissues, principally the periodontal and gastrointestinal mucosa. In these tissues, H_2S and mercaptans in the nanomolar range have been shown to inhibit DNA synthesis, suppress collagen synthesis, increase the intracellular degradation of newly synthesized collagen, and increase mucosal permeability (Tables 14 and 15).

2.0 EFFECTS OF HYDROGEN SULPHIDE ON HEALTH ENDPOINTS OR ORGAN SYSTEMS

2.1 CANCER

2.1.1 Human and Animal Studies

There were two animal studies during the past decade that directly related H_2S exposure to cancer occurrence (see Table 1). The first study examined rats in a subchronic inhalation study and suggested that H_2S exposure was associated with a slight, but increased, risk for cancers of the respiratory tract (Brenneman et al., 2000).

A few epidemiological studies during the past decade examined the relationship between exposure to environments containing H_2S and other compounds and occurrence of cancer (Table 1).

Human populations in geothermal areas, salt marsh areas, sewer treatment plants, pulp and paper plants, sulfide mines and petrochemical refineries have been examined (see Table 1). There appears to be a consistent increased risk of respiratory tract cancers found in populations exposed to sewer gas (Friis et al., 1999; Lafleur and Vena, 1991). In a cohort of 711 sewer workers, a significant increase of cancer of the nose and nasal sinuses (Standardized Incidence Ratio [SIR] 12; 95%CI 1.5 - 44) was found, although this finding was based only on two cases (Friis et al., 1999). The increase risk of laryngeal cancer in sewer workers (Standardized mortality ratio [SMR] 7.93; 95% CI 1.59 - 23.96) reported by Lafleur and Vena (1991) was thought to be work-related. Sewer workers were also reported to be at increased risk for other cancers such as stomach, leukemia, lymphatic, (Friis et al., 1999; Betemps et al., 1994; Andersson et al., 2001), prostate (Friis et al. 1999), brain (Andersson et al., 2001), kidney (Friis et al., 1993), and liver (Lafleur and Vena, 1991; Andersson et al., 2001). Statistically significant increased rates of nasal cancer, and slightly increased rates (although not statistically significant) of trachea, bronchus and lung cancer have been reported among Maori women living in Rotorua, a geothermal area of New Zealand (Bates et al., 1998).

In a study of 23,718 male pulp and paper workers, significant increases in risk were found for lung cancer among both short- and long-term employees; highest for sulfite mill workers (Langseth and Anderson, 2000). Another study (Andersson et al., 2001) revealed increased risk of death due to cancer for sulfate mill workers. Cancer risks were significantly associated with work duration for male workers in Kraft paper mills (Band et al. 1997). This cohort study consisted of 30,157 male pulp and paper workers. Significantly increased risk of mortality (between 1970 and 1984) due to malignant neoplasms and lung cancer was found in 201 men working in pulp and paper production (Solet et al., 1989; Table A1; Appendix I). Elevated risks were also found for lymphopoietic system cancer (Schwartz, 1998; Solet et al., 1989) and cancer of the large intestine (Solet et al. 1989).

In a study of Finnish sulfide ore miners (14,782 person years) compared to the general male Finnish population, a two-fold increased risk of lung cancer was observed (Ahlman, 1991). Of the 10 lung cancer cases, five worked in the zinc mine and three were diesel-powered ore train operators. The findings were believed to be associated with the radon, crystalline silica, and diesel exhaust gases in the mine. Sulfide exposure was not considered a possible contributing agent.

In petrochemical refinery workers, increased risks were found for neoplasm of uncertain behavior and unspecified nature of the eye, brain, and nervous system, SMR 254 [95%CI 122 - 466] (Gamble et al., 2000), and for lymphatic and hematopoietic tissue, SMR 122 (95% CI 101 - 146] and neoplasm of uncertain behavior and unspecified nature of the eye, brain, and nervous system, SMR 240 [95%CI 128 - 410] (Lewis et al., 2000) [3],

Previous studies (see Table A1, Appendix I) reported greater incidence of cancer of people living in salt marsh areas (Hitchcock, 1979; Harrington et al., 1978; Voors et al. 1978) and workers in the fishing industry (Gottlieb et al., 1979). The single report of increased risk of testicular cancer found in a hospital-based case-control study of oil and gas workers (Mills et al., 1984) has not been replicated. Although few studies appear to have specifically looked for effects on testicular cancer, it is of interest that Dorman et al. (2000) observed in their rat study, a higher incidence, but not statistically significant, of testicular tubular degeneration in male rats from the 80 ppm exposure group (42%) as compared to the control animals (17%).

Several other (anecdotal) reports, although most likely coincidental findings, were located. J. Round (1992) had a gilt in gestation exposed during the Lodgepole blowout that developed lymphosarcoma. The provincial laboratory pathologist indicated that it was 'very unusual to have a case of porcine (swine) lymphosarcoma in Alberta'. A member of the Bocock family, living 6 miles west of a sour gas processing plant developed a low-grade lymphoma (Nikiforuk, 2001; p. 98). An organic farmer, whose water was so contaminated that you could set it on fire, lived near a sour gas well owned by Rigel. The well had vented and flared by his house for seven days in 1990. He died of lymphatic cancer in 2001 (Nikiforuk, 2001; p. 258).

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[3] Neither of the Gamble or Lewis papers characterise the petroleum industry from which their cohort derives as 'upstream' or 'downstream'. While these studies do not specifically mention H2S as a hazard, they also do not specifically mention any of the other hazardous agents at refinery and petrochemical plants that the workers may have been exposed to. This is a common shortfall in many published epidemiology studies. Yet in petrochemical refinery processes, it is known that hydrogen sulfide is present:
i) as a by product of many industrial operations, eg, coking and the hydrodesulfurization of crude oil and of coal [Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed., Volumes 1-26. New York, NY: John Wiley and Sons, 1978-1984, p. V26 1131 (1985)
ii) as a component of crude petroleum. [Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996. 823]
iii) the majority of occupational exposures to hydrogen sulfide resulted from its occurrence in petroleum, natural gas, soil, sewer gas & as byproduct of chem reactions. [American Conference of Governmental Industrial Hygienists, Inc. Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Volumes I,II, III. Cincinnati, OH: ACGIH, 1991. 786]

2.1.2 *In vitro Studies*

With the exception of one study of the genotoxicity of landfill gas (Sadowska et al., 1999), there appears to be a lack of studies investigating the effects of H_2S on cancer development during the past decade. Earlier studies have been summarized in Table A2, Appendix I. Perhaps related, sex chromosome loss and non-disjunction were reported in *Drosophila melanogaster* continuously exposed to 99 ppb methyl mercaptan gas for 6 months (RTECS, 2000).

The United States Environmental Protection Agency (US EPA) states however that "this substance/agent [hydrogen sulfide] has not undergone a complete evaluation and determination under US EPA's Integrated Risk Information System (IRIS) program for evidence of human carcinogenic potential" (US EPA IRIS, 1998).

2.1.3 *Limitations and Gaps*

The majority of the studies described in this section involved human subjects exposed to other carcinogens, and few were controlled for co-exposures. Few studies had validated exposure measures. These factors severely limit the detection of causal relationships. Yet, during the past decade, studies from diverse settings suggest, but have not confirmed, that H_2S has carcinogenic potential. This justifies the need for additional studies to more clearly define the relationship between cancer and H_2S exposure. The findings of Brennemen et al. (2000) strongly support further studies examining the nasal and upper respiratory tract and lymphoid system, among diverse H_2S-exposed groups. Several related factors that may be secondary to H_2S exposure also support the need for a research program into carcinogenic effects: i) the rapid transformation of biogenic sulfur to sulfate and sulfuric acid mists in polluted air (Warneck, 1988); ii) the International Agency for Research on Cancer's (IARC) designation that strong inorganic acid mists containing sulfuric acid are carcinogenic to humans (IARC, 1992); and iii) activation of protein kinase c by reactive oxygen species (Knapp and Klann, 2000) produced as a consequence of antioxidant enzyme and cytochrome c oxidase inhibition by H_2S.

It may be difficult to find populations exposed solely to H_2S, therefore additional human epidemiological studies may be limited. Since human populations often experience mixed exposures (carcinogens and other substances), long-term studies in animal species, in addition to rat, and supplementary *in vitro* studies are warranted. These should be designed to assess the carcinogenic potential of H_2S per se, as well as in combination with other substances. Examples include: mercaptans and endotoxins (i.e. in the pulp industry, farming and animal waste handling industry), ammonia (in waste handling operations), other metal-containing particles (in sulfide mining), and more oxidized sulfur species (in pulp and paper plants and in ambient air around some industrial sources).

Table 1 Cancer Risks Identified in Human, Animal and *In vitro* Studies

Reference / Study Design	Key Findings
Andersson et al. (2001) A case-referent study of 2480 men aged 40 - 75 at death during 1960 - 1989 in the parishes around 4 sulfate mills was undertaken. Exposure assessment was based on information found in the personnel files and work categories were created.	Among all sulfate mill workers, increased risk was found for death from lung cancer (Odds ratio [OR] 1.6; 90% CI 1.1 to 2.3), pleural mesotheliomas (OR 9.5; 90% CI 1.9 - 48), for brain tumors 2.6 (1.2 to 5.3) and liver or biliary tract cancer 2.3 (1.0 - 5.2). Increase mortality from leukemia was also found among soda recovery plant workers OR 5.9 (2.6 - 13) and bleaching plant and digestor house OR 2.8 (1.0 - 7.5). The increased risk of dying from lung cancer and pleural mesotheliomas was, according to the authors, due probably to asbestos exposure.
Langseth and Andersen (2000) Male pulp and paper workers (23,718) employed continuously for 1 year were followed from 1953 - 1993.	An excess incidence of lung cancer was found among both short- (Standardized incidence ratio [SIR] 1.5; 95% CI 1.13 - 2.03) and long-term (SIR 1.2; 95% CI 1.09 - 1.34) employees. The risk was highest for sulfite mill workers (SIR 1.5; 95% CI 1.09 - 1.99). An increased risk of malignant melanoma was also found (SIR 1.3; 95%CI 1.04 - 1.60).
Brenneman et al. (2000) In a subchronic study, rats were exposed to H_2S (0, 10, 30, or 80 ppm) for 6 hr/d, 7 d/wk for 10 weeks.	Animals exposed to 30 ppm had mild (25 - 50% reduction in the normal thickness of the olfactory neuron layer) to moderate (50 - 75% reduction) olfactory neuron loss and basal cell hyperplasia in the lining of the dorsal medial meatus and the dorsal and medial regions of the ethmoid recess.
Gamble et al. (2000) A cohort of 6238 retirees from three refinery/petrochemical plants was analyzed for death from all causes.	Increased standardized mortality ratios [SMR] were found for neoplasm of uncertain behavior and unspecified nature of the eye, brain, and nervous system, SMR 254 [95%CI 122 - 466].
Lewis et al. (2000) Mortality in a cohort of 19,075 active and terminated workers at 3 refinery/petrochemical plants was compared to national and state mortality rates for all causes.	Increase SMRs were found for: i) lymphatic and hematopoietic tissue, SMR 122 (95% CI 101 - 146]; ii) neoplasm of uncertain behavior and unspecified nature of the eye, brain, and nervous system, SMR 240 [95%CI 128 - 410].
Sadowska et al. (1999) Genotoxicity of air surrounding a landfill in Belgium was measured using the *Tradescantia* micronucleus assay.	7.38% micronuclei were found in samples taken on the landfill, 6.35% in the immediate surroundings (less than 100 m), and 5.5% at a greater distance. Higher frequencies were found for a main street in a nearby town (7.39%) and a few meters from a nearby busy highway (10.36%).
Friis et al. (1999) The cancer incidence in a cohort of 711 Swedish sewage workers at 17 plants employed for at least 1 year during the years 1965 - 86 was compared with the expected rate. Sewage exposure was based on a qualitative classification.	The total cancer incidence was not significantly increased (SIR 1.2; 95% CI 0.92 - 1.5). A significant increased in prostate cancer was found (SIR 1.6; 95% CI 1.0 - 2.5). Based on two cases, a significant increase of cancer of the nose and nasal sinuses (SIR 12; 95%CI 1.5 - 44) was found. Stomach cancer incidence was also increased (SIR 2.3; 95% 0.99 - 4.5). No relation between cancer incidence and level of sewage exposure was found.

Andersson, E., T. Nilsson, B. Persson et al. (1998) A case-referent study was undertaken to investigate whether sulfite workers showed increased mortality from asthma, COPD or certain malignancies among 780 men aged 40 - 75 years at death who died between 1960 and 1989 in parishes surrounding 3 sulfite mills.	Among the sulfite workers, an increased mortality from asthma (OR 2.8; 90%CI 1.1 - 6.8) and brain tumors (OR 3.3; 90%CI 1.2 - 8.9) was found. Two of the 5 exposed brain tumor cases had worked in the wood room and 1/5 worked in the bale or finishing room. Mortality due to lung cancer was increased, although not significantly (OR 1.4; 90%CI 0.7 - 2.6) and there was reduced mortality from stomach cancer (OR 0.4; 90%CI 0.2 - 0.9).
Bates et al. (1998) Cancer registry and hospital discharge data to compare rates of cancer and disease in Rotorua, New Zealand with the rest of New Zealand. Rotorua sits on a geothermal field that has continuous ambient low-level H_2S (median conc 20 μg/m3; 14 ppb) and mercury.	There were elevated rates for bronchus and unspecified lung (SIR 1.45; 95% CI 1.13 - 1.84), and for nasal cancers (SIR 3.17; 95% CI 0.85 - 8.11) however the SIR for nasal cancers was based only on four cases. There were elevated rates for Maori women for cancers of the trachea, bronchus, and lung (SIR 1.48; 95%CI 1.03 - 2.06), not explained by smoking rates.
Matanoski, G.M. et al. (1998) Death rates of a cohort of 63,025 pulp and paper workers from 51 mills from 23 US corporations employed 10 or more years between 1970 and 1991 were compared with US rates.	Reduced risks of death from all causes (SMR 0.74) and all cancers (SMR 0.84) were found. Increased lung cancer risks (relative risk [RR] 1.35; 95% CI 1.04 - 1.75); brain cancer (RR 2.35; 95% CI 0.74 - 7.53); liver (RR 2.37; 95% CI 0.57 - 9.88) were associated with the Kraft process. Increased brain cancer risks (RR 2.33; 95% CI 1.38 - 3.93) and lymphomas (RR 1.68; 95%CI 1.03 - 2.72) were associated with 'other chemical pulping'. No significant risk increases were found for the sulfite process.
Band et al. (1997) A cohort of 30,157 male pulp and paper workers in British Columbia, Canada were studied. The cohort consisted of 20,373 workers in kraft mills only, 5249 in sulfite mills only, and 4535 in both kraft and sulfite mills. Mortality rates of all workers with at least one year of employment on Jan 1, 1950 or thereafter until Dec 31, 1992 were compared with the Canadian male population.	Cancer risks were significantly associated with work duration and time from first employment of 15 years of more were found for the total cohort for cancers of the pleura (SMR 3.61; 90%CI 1.42 - 7.58) for kidney (SMR 1.69; 90%CI 1.13 - 2.43) and the brain (SMR 1.51; 90%CI 1.03 - 2.16). Cancer risks were significantly associated with work duration and time from first employment of 15 years of more were found for workers in kraft mills only for kidney cancer (SMR 1.92; 90%CI 1.04 - 3.26) and for workers in sulfite mills only, Hodgkin's disease was increased (SMR 4.79; 90%CI 1.29 - 12.37). For workers ever employed in both kraft and sulfite mills, esophagus cancer risks were increased (SMR 1.91; 90% CI 1.00 - 3.33). Increased risks for cancer of the eye (SMR 3.41; 90%CI 1.16 - 7.79), although based only on 4 cases, were also found.
Andersson, Hagberg, Nilsson et al. (1996) A case-reference mortality study among sulfate mill workers.	Increased risk for brain tumors is reported (OR 2.6; 95% CI 1.1 - 6.1).
Betemps et al. (1994) Mortality among sewage treatment plant workers born in different countries was evaluated in a retrospective cohort. Death certificates of white males employed for at least 6 months during the 1960s at the Metropolitan Water Reclamation District of Chicago were obtained.	Compared to the US white male population, workers born outside the US had significantly increased mortality from stomach cancer (SMR 4.27; 95% CI 2.06 - 8.87), leukemia (SMR 3.67; 95% CI 1.47 - 9.07) and lymphatic cancer (SMR 2.74; 95%CI 1.35 - 5.53). The US-born group had higher mortality from benign tumors (SMR 2.87; 95%CI 1.34 - 6.16).

Friis et al. (1993) Mortality and cancer incidence among 656 Swedish men who worked in sewage treatment pants for at least one year at any one of 17 sewage treatment plants between 1965 - 86 were compared with the general Swedish population in a retrospective cohort design.	For all cancers, the combined mortality (SMR 1.08; 95%CI 0.68 - 1.67) and morbidity (SMR 1.02; 95% CI 0.72 - 1.28) were comparable with the general population. There were increased incidences for brain tumors (SMR 2.19; 95% CI 0.45 - 6.38) for gastric cancers, (SMR 2.73; 95% CI 1.00 - 5.94) and for renal cancers (SMR 1.68; 95% CI 0.35 - 4.90). Renal cancer had a significant positive relation with the weighted sum of employment, but no relation was found for brain tumors or gastric cancer.
Halasova et al. (1993) Cytogenetic analysis of peripheral blood lymphocytes was done on 2 groups of men, 30 in each, who worked in a cellulose paper works plant (in the regeneration and caustification area) and an industrial water disposal plant. Two control groups, 30 in each, were matched for age, smoking and alcohol consumption.	Compared to controls (2.87% in a the town control group; 1.67% in the medical student control group), increased clastogenic effects on lymphocyte chromosomes were found in both workplaces. The industrial wastewater disposal plant workers had 5.97% and the cellulose paper workers had 5.07% of aberrant cells.
Lafleur and Vena (1991) A retrospective cohort study of cancer mortality among 487 white male sewage treatment plant workers in Buffalo, New York, employed between 1950 and 1979 was undertaken. Comparisons were made with the general white male population of the entire US. Sewer exposures were classified by job titles.	Mortality from all cancers among exposed sewer workers was slightly higher than that of the general population (SMR 1.19; 95% CI 0.79 - 1.7). Among the exposed, the risk for death from cancer of the larynx (SMR 7.93; 95%CI 1.59 - 23.96) and liver (SMR 5.4; 95% CI 1.1 - 16.05) was significantly higher than among controls. The highest exposed group had a higher directly adjusted death rate (rate ratio 1.64) from all neoplasms combined compared to all other workers. Review of the medical and occupational histories of the cases suggested that the laryngeal cancer was possibly work-related while the liver cancer was not.
Ahlman et al. (1991) Lung cancer mortality was studied among sulfide ore miners (14,782 person years) in north Finland between 1965 and 1985. All lung cancer deaths in the township of Outokumpu were identified through death certificates and compared with company personnel records. Occupational history, smoking habits and alcohol consumption was obtained from workers or next of kin. The worker death rates were compared to the regional rates.	Compared to Finnish males, a 2-fold increased in lung cancer was found, and compared to North Karelian males, a 40% increased risk was found. The lung cancers were found predominately from the zinc mine and diesel powered ore trains. The authors attributed the increased risk to exposure to radon daughters, and combined effects of silica dust and diesel exhaust gases.
Wingren et al. (1991) A case-referent study of 4070 pulp and paper mill workers deceased between 1950 and 1987 in Sweden was undertaken. The subjects were identified from the register of deaths and burials in six parishes.	A significantly increased mortality was seen for diabetes mellitus (OR 1.3; 90%CI 1.0 - 1.7) and for secondary tumors of the lung and liver (OR 8.1; 90% CI 2.7 - 24.4) among the pulp and paper mill workers. When the analysis was restricted to men 40 - 79 years of age, the odds rations increased for malignant lymphomas, (OR 2.1; 90% CI 0.8 - 5.6) and leukemias (OR 1.2; 90% CI 0.6 - 2.5); also the risk for secondary tumors of the lung and liver was increased (OR 22.1; 90% CI 6.1 - 80.5). Indications of excess risks were also found for obstructive lung disorders, pulmonary emboli, accidents and pneumonia, as well as for malignant lymphomas, leukemia, and cancers of the pancreas and stomach. In the only parish where only the sulfite process was used, digestive tract cancer, especially of the rectum, was found in excess.

2.2 CARDIOVASCULAR EFFECTS

Many alterations in cardiovascular function have been associated with H_2S exposure (Tables 2.1 and 2.2). Studies during the past decade have also provided a better understanding of the cardiovascular risks associated with H_2S. These studies add to the previous literature documenting both short and long-term cardiovascular effects of H_2S in humans and animals (see Tables A3 and A4 in Appendix I). Prior to 1990, with one exception, effects such as increased arterial blood pressure, decreased heart rate and changes in cardiac rhythms, and less frequently bradycardia and tachycardia, were reported.

2.2.1 Human and Animal Studies

With the exception of sewer workers in Denmark (Friis et al., 1993), several large epidemiological studies of H_2S exposed groups, including workers in sewers (Betemps et al., 1994), sulfate mills (Jappinen and Tola, 1990), pulp and paper mills (Hammar et al., 1992), sulfide ore mines (Ahlman et al., 1991) and petrochemical refineries (Lewis et al., 2000, Gamble et al., 2000) have revealed slight increased risks for cardiovascular disease. Among mill workers exposed to H_2S and organic sulfides, from a total of 4179 person-years of follow-up, compared to the national death rates, an excess of cardiovascular deaths was observed (Standardized Mortality Ratio [SMR] 150; 95%CI 105 - 206), due principally to coronary deaths (SMR 150; 95%CI 97 - 222) (Jappinen and Tola, 1990). Common risk factors could not explain the findings in this cohort.

Significantly increased incidence of circulatory system diseases was found (Standardized Incidence Ratio [SIR] 1.05; 95%CI 1.02 - 1.07) for citizens living in a geothermal area where median H_2S concentrations were measured at 21 ppb (Bates et al., 1998). Risk estimates for hypertensive disease (SIR 1.15; 95%CI 1.00 - 1.32); other heart disease (SIR 1.06; 95% CI 1.00 - 1.13); diseases of arteries, arterioles and capillaries (SIR 1.17; 95%CI 1.07 - 1.28); and diseases of veins and lymphatics and other circulatory diseases (SIR 1.22; 95%CI 1.07 - 1.28) were all slightly increased.

In a study of 597sulfide ore miners in Finland involving 14,782 person years of follow-up, Ahlman et al. (1991) found increased risk for cardiovascular diseases. Compared to the general male population, a two-fold excess mortality due mainly to ischemic heart disease was found. Forty-five percent of the 44 miners in the age range of 45 to 64 who died from ischemic heart disease had been drillers or chargers (12 were copper miners and 8 were zinc miners). Also, a two-fold increased risk of ischemic heart disease was found in the subgroup of copper miners. The authors proposed that the increased mortality from ischemic heart disease among drillers and chargers might partly be due to exposure to nitroglycerin as well as mental and physical stresses. There is, however, the potential that crushed sulfide ores emitted H_2S or metal-sulfide particles that resulted in chronic exposure of these worker subgroups.

Animals acutely exposed to H_2S have exhibited cardiac changes such as arrhythmias (Kohno et al., 1991), sinus tachycardia (Tvedt et al., 1991a; Horowitz et al., 1997; Schneider et al., 1998) and, in humans, elevated cardiac enzymes (Gregorakos et al., 1995; Tanaka et al., 1999).

Increased blood lactate following exposure reported by several investigators (Seredenko et al., 1991; Bhambhani et al., 1991, 1995, 1996, 1997; Pach et al., 1996) indicates a switching of aerobic to anaerobic metabolism, which if sustained for extended periods may lead to cell death. Cells with high ATP production requirements such as cardiac and nervous tissues are especially vulnerable (Voet, 1999; p. 523). For cardiac tissue, after 20 minutes of occlusion, over 60% of the ATP and all cellular glycogen is used up, and the amount of lactate is 12 times the normal aerobic level (Bremner, 1984; p. 495). Hydrogen sulfide has multiple effects on glucose metabolism (Khan, 1989), inhibiting: i) pyruvate dehydrogenase (the enzyme needed to make feedstock Acetyl-CoA for the citric acid cycle); ii) cytochrome c oxidase the terminal enzyme in the electron transport chain; iii) citrate synthase (the first enzyme in the citric acid cycle); and iv) aconitase (the second enzyme in the citric acid cycle). The combined effects would be expected to reduce NADH and $FADH_2$ generation. As the most crucial regulators of the citric acid cycle are acetyl Co-A, oxaloacetate and NADH (Voet, 1999; p. 486), reduced production of these substrates would be expected to shut the cycle down[4].

Cytosolic enzymes in the plasma and extracellular fluid were reported by many investigators (See Tables 11, 26, and 27). These findings are indicative of cellular damage. At high H_2S concentrations, leaking was observed in lungs (Green et al., 1991; Khan et al., 1995) and plasma of exposed animals (Edwards, 1992; Church, 1992). Leaking was also reported in lung fibroblasts *(in vitro)* at low concentrations (0.01 uM) of sodium sulfide (Hayden et al., 1990a). Elevated cardiac enzyme levels following H_2S exposure (Gregorakos et al., 1995; Tanaka et al., 1999) are similar in magnitude and timing as those found after a myocardial infarction. Bremner (1984) stated *"the distinguishing point between reversible and irreversible ischemic injury is considered to be the inability of the cell to maintain membrane integrity. It is the release of cardiac enzymes that is particularly significant in the evaluation and confirmation of irreversible ischemic injury. The enzymes that are released after ischemic irreversible injury are soluble enzymes such as creatine kinase, aspartate aminotransferase, and lactate dehydrogenase."* The elevated cardiac enzymes found in plasma of H_2S exposed workers (Gregorakos et al., 1995; Tanaka et al., 1999), may provide an explanation for the subsequent increased risk for myocardial infarction and cardiovascular disease reported.

2.2.2 Limitations and Gaps

Studies of H_2S-exposed workers have consistently shown slight increased risks for cardiovascular disease. Unfortunately, few studies controlled for other known cardiovascular risk factors, or collected good exposure data. It is therefore difficult to assess what proportion of the risk is related to H_2S exposure. The presence of elevated blood lactate levels observed after only a 30-minute exposure to 5 ppm H_2S or cardiac-specific enzymes present in the blood after acute exposures suggest that energy production and cellular integrity are disrupted. Understanding the overall effects is complicated by the interrelationship between sulfur metabolism and other known cardiovascular risk factors, e.g. the role of homocysteine, elevated cholesterol levels in chronically exposed rats (Hayden et al., 1990c) and altered lipid and carbohydrate metabolism in sulfur dioxide exposed rats (Lovati et al., 1996).

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[4] Inhibition of the citric acid cycle would shift energy production to glycosis, however as discussed in Sections 7.6.1 and 10.4, quite a few enzymes in this pathway are also inhibited by H_2S.

If one or more of the homocysteine metabolizing pathways of the methyl cycle (Fig 1; Appendix III) are inhibited due to enzymatic defects or vitamin deficiencies (Vitamin B_6, B_{12} and folate), homocysteine accumulates thereby producing increased plasma homocysteine levels (Ueland et al., 1992). Slight increases in homocysteine levels have recently been shown to be an independent risk factor for development of cardiovascular disease[5] (McCully, 1999; Refsum et al., 1998; Booth et al. 2000). Homocysteine significantly increased IL-1 induced nitric oxide (NO) synthesis and nitrite production in a dose-dependent manner in vascular smooth muscle cells (Ikeda et al., 1999). The apparent ability of H_2S to relax smooth muscle in conjunction with nitric oxide generators (Hosoki et al., 1997; Zhao et al., 2001) suggests that H_2S may play a key role in modulating vascular tone and endothelial cell function. Important and strong interactions between trace gases (H_2S and NO) in biological systems may be involved in the blood pressure changes observed in exposed animals (Baldelli et al., 1993) and humans (Table 2). Thus, the effects of exogenous H_2S on vascular tone should be examined in more detail.

Further studies should also be undertaken to assess the effects of H_2S and other reduced sulfur gases on cytokine production. Recent work suggests that cytokines, such as interleukin-1, directly affect mitochondrial function. Ratkay et al. (1995) reported that methyl mercaptan (CH_3SH) induced interleukin-1 (IL-1) production in monocytes *in vitro*. Tonsillar mononuclear cells incubated with 7 ppb CH_3SH in 5% CO_2/95% air headspace for 48 hours enhanced IL-1 production two-fold, similar to that found with 2-mercaptoethanol. IL-1 is one of the pro-inflammatory cytokines that can increase NO synthesis by elevating the expression of inducible nitric oxide synthase. NO was shown to cause dose-dependent inhibition of mitochondrial respiration in macrophages as well as in smooth muscle cells by inhibiting Complex I and II activity (Geng, et al., 1992). Nitric oxide synthase was induced, increased nitrite levels were found and mitochondrial activity significantly inhibited by IL-1 in cardiac myocytes (Oddis & Finkel, 1995). Tatsumi et al. (2000) recently reported that IL-1-induced NO production, which was dose-dependent, lowers energy production and myocardial contractility through a direct attack on mitochondria. The mitochondrial enzymes NADH-CoQ Reductase (Complex I) and Succinate-CoQ Reductase (Complex II) were each inhibited 20% following a 24-hour incubation of cardiac myocytes with 10 ng/mL IL-1[6]. They also found that addition of cyanide, a Complex

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[5] As given in Booth et al. (2000) "Over 30 case-control studies have compared total homocysteine (tHcy) levels between coronary artery disease (CAD) patients and healthy control subjects. Patients with CAD had significantly higher fasting plasma tHcy levels in 22 of 27 studies (odds ratio [OR] 1.2 - 10.9 after adjustment for other cardiac risk factors). Levels after methionine load were also higher in patients with CAD in 8 of 9 studies (adjusted OR 1.3 - 7.6). Measurement of serum levels yielded similar results. Moreover, 2 meta-analysis of retrospective data confirmed these findings: the odds ratios of CAD associated with elevated plasma tHcy levels were 1.7 (95% CI 1.5 - 1.9) and 6.14 (95% CI 2.74 - 13.73)".

[6] Recent work by Davey et al. (1998) indicates that Complex I is the rate-limiting step in the chain and most likely to be the main regulator of oxidative phosphorylation. These investigators examined the relative contribution of individual mitochondrial chain complexes to the control of NAD-linked substrate oxidative phosphorylation in synaptic mitochondria. They found that before major changes in the rates of oxygen consumption and ATP synthesis occured, Complex I, III, and IV activities were decreased by 25, 80 and 70% respectively.

IV respiratory inhibitor, to the IL-1-treated cells significantly worsened ATP depletion as compared to IL-1 alone.

These findings are relevant to H_2S or methyl mercaptan exposures. Both gases are small, uncharged molecules, able to penetrate cells easily, and are able to increase membrane permeability at nanomolar and micromolar concentrations (Ng and Tonzetich, 1984; Hayden et al., 1990a) allowing leakage of cellular constituents into the extracellular spaces in a dose-dependent manner. Leakage of cellular constituents and irritation of sensory afferent C-fibers via substance P induces IL-1 production (Rothwell and Hopkins, 1995). Thus in the presence of IL-1, H_2S could have even greater deleterious effects on cellular respiration, affecting tissues with high energy requirements such as the myocardium and nervous tissue.

Since the number of adults with cardiovascular disease in the general population is significant, it is important to have a better understanding of the effects of H_2S on cardiovascular functions, as well as sulfur metabolism, the role of oxidants and anti-oxidants, and the interactions of diet and inhaled sulfur compounds.

2.3 ENDOCRINE EFFECTS

There have only been a few studies conducted during the past decade that have explored the relationship between H_2S and the endocrine system (Tables 4, 5), however reports describing effects on carbohydrate metabolism, smooth muscle function and core temperature control were located.

Several reports have shown effects on carbohydrate metabolism, diabetes in particular, following sulfide or thiol exposure. Increased risk of diabetes mellitus has been reported in a cohort of pulp and paper workers (Wingren et al., 1991) and in workers in sulfite digestion and paperboard manufacturing (Beelen et al., 2000). These studies are in agreement with earlier studies by Schwartz (1988), Henneberger et al. (1989), Thomas et al. (1985), and Elebekova et al. (1976) (see Table A5 in Appendix I). Mild cranial diabetes was reported in acutely exposed individuals (Watt et al., 1997). In an animal study, hyperglycemia was observed in rat dams exposed to 20-75 ppm H_2S from day 1 of gestation to day 21 post-partum (Hayden et al., 1990c). Hyperglycemia during pregnancy is a risk factor for intrauterine deaths, congenital malformations, and perinatal mortality and morbidity in fetuses (Russell, 1984). As a low molecular weight dimer with multiple disulfide bonds, acting at nanomolar concentration in the circulation, insulin is susceptible to disruption by H_2S (Jocelyn, 1972).

Several stages of thyroid metabolism such as energy production or hormone response may be affected by H_2S which may contribute to the fatigue that is often reported following H_2S exposure (See tables in nervous system section) and has been reported in animals (See Tables A5, A6; Appendix I). Several peroxidases are inhibited by sulfide (cited in Beauchamp et al., 1984). Since there is structural similarity among mammalian peroxidases (Thomsen et al., 2000), it is likely that H_2S also inhibits thyroid peroxidase. Also, given that type I iodothyronine deiodinase has cysteine at its active site and is inhibited by dithiothreitol (Sun et al., 1997), H_2S

Table 2 Cardiovascular System Effects: Human Studies

Reference \ Study Design	Key Findings
van Aalst et al. (2000) Case report of 2 workers exposed to H_2S	For both workers, ECG showed sinus tachycardia; blood pressures were 160/70 and 160/120 mm Hg.
Gamble et al. (2000) A cohort of 6238 retirees from three refinery/petrochemical plants was analyzed for death from all causes. Standardized mortality ratios (SMRs) were calculated.	Increased SMRs were found for ischemic heart disease, SMR 113 [95% CI 108 - 118]; and hypertension with heart disease, SMR 132 [95%CI 98 - 172].
Lewis et al. (2000) A cohort of 19,075 active and terminated workers at three refinery/petrochemical plants was compared to national and state mortality rates for all causes.	Increased SMR was found for hypertension with heart disease, SMR 123 [95%CI 88 - 166].
Tanaka et al. (1999) Case report of 3 workers exposed to H_2S.	Creatine phosphokinase was elevated in all three patients (224, 546, 946 IU/L; normal <160 IU/L) measured on the second and third days after admission.
Bates et al. (1998) A retrospective epidemiologic study using hospital discharge data from 1981 - 1990. Rates of disease in Rotorua, New Zealand were compared with the rest of New Zealand. Rotorua sits on a geothermal field that has continuous ambient H_2S (median conc 20 µg/m3; 14 ppb) and mercury.	A significant increase in incidence of circulatory system diseases was found (Standardized Incidence Ratio [SIR] 1.05; 95%CI 1.02 - 1.07). Further classification showed increased incidences of: hypertensive disease (SIR 1.15; 95%CI 1.00 - 1.32); other heart disease (SIR 1.06; 95% CI 1.00 - 1.13); diseases of arteries, arterioles and capillaries (SIR 1.17; 95%CI 1.07 - 1.28); and diseases of veins and lymphatics and other circulatory diseases (SIR 1.22; 95%CI 1.07 - 1.28).
Schneider et al. (1998); Anonymous (1993) Case report of a construction worker who was exposed to H_2S from a wetland pit. Measurements at the site four hours after the event indicated concentrations of 22 ppm H_2S at the surface of the water.	The worker, unconscious upon admission to hospital, had a heart rate of 122 beats/min, sinus tachycardia, blood pressure of 130/65 mm Hg, respiration of 16 breaths per minute, temperature was 101.2F and carboxyhemoglobin was 14.9%.
Horowitz et al. (1997) Case report of two cases of poisoning from a fungicide, calcium polysulfide. The first involved a 23-year old woman who ingested a half a cup of 26% calcium polysulfide (dose estimated at 563 mg/kg).	Among many findings, the physical exam of the women revealed a strong smell of rotten eggs. An initial ECG revealed a sinus tachycardia. Seven hours later, an ECG showed a sinus tachycardia at 170, and other findings consistent with a myocardial infarction. Eleven hours post-ingestion, the patient sustained a cardiac arrest from which she could not be resuscitated.
Bhambhani et al. (1997) 15 men and 13 women completed two 30-minute exercise sessions 50% of their maximal oxygen uptake while breathing medical air or 10 ppm H_2S (blinded to treatment) and ECG and blood pressure were monitored. Arterial and finger-prick blood samples were obtained during the final minute of exercise. Muscle biopsies were obtained immediately after exercise.	A significant decrease in oxygen uptake and an increase in blood lactate were found in both men and women. No significant changes in arterial blood parameters were observed. Muscle lactate, LDH, citrate synthase and cytochrome oxidase was not significantly affected however there was a tendency for muscle lactate to increase and citrate synthase to decrease with H_2S exposure.

Bhambhani et al. (1996) 25 young healthy volunteers (13 men, 12 women) exercised at 50% of their maximal power for 30 minutes while breathing 0 or 5 ppm H_2S. Immediately after exercise, muscle biopsies were obtained and tested for markers of anaerobic metabolism: lactate, lactate dehydrogenase, citrate synthase, and cytochrome oxidase activity.	A decrease in muscle citrate synthase in men was observed following a 30-minute exposure to 5 ppm H_2S. There was also a tendency, although not significant, for lactate and LDH to increase and cytochrome oxidase to decrease.
Gregorakos et al. (1995) Case report of 8 sewer workers exposed to high concentrations of H_2S	Various ECG alterations were found: elevation of ST segment consistent with acute change of myocardial infarction; right bundle branch block and left anterior hemiblock; and depolarization changes in the anterior and lateral wall. Creatinine phosphokinase MB, a sensitive indicator of cardiac cell damage, was elevated (> 45 U; normal range < 18U) in all 4 patients that survived the initial episode. Lactate dehydrogenase levels were abnormal for all four patients (actual values were not given, nor was any isozyme analysis done). Of four patients that survived the acute episode, one patient subsequently died of cardiogenic pulmonary edema and circulatory failure, the other 3 left the hospital in apparent good health. One left the hospital with a temporary pacemaker, and one of the other two died of a myocardial infarction, without any previous history concerning cardiac-related disease, after two months.
Betemps et al. (1994) Mortality among sewage treatment plant workers born in different countries was evaluated in a retrospective cohort. Death certificates of white males employed for at least 6 months during the 1960s at the Metropolitan Water Reclamation District of Chicago were obtained.	The US-born group had higher mortality from arteriosclerotic cardiovascular diseases (SMR 1.15; 95%CI 1.06 - 1.24).
Bhambhani et al. (1994) 13 young healthy male and female volunteers were exposed to 5 ppm H_2S for 30 minutes while undergoing submaximal exercise.	No changes in oxygen uptake, carbon dioxide production, respiratory exchange ratio, heart rate, blood pressure, rate pressure product, arterial oxygen, carbon dioxide, pH, % hemoglobin saturation, perceived exertion, or other parameters were found.
Friis et al. (1993) Mortality and cancer incidence among 656 Swedish men who worked in sewage treatment plants were compared with the general Swedish population in a retrospective cohort design.	Mortality from cardiovascular disease was lower among employees in the sewage treatment plants (SMR 0.61; 95% CI 0.39 - 0.91)
Hammar et al. (1992) In a case-referent study, cases of myocardial infarction (36,602) were identified from both hospital discharge and death records. Two referents for each case were randomly selected from the study base. Information about occupation was obtained from two consecutive censuses.	Increased incidence of myocardial infarction was observed after controlling for age and socioeconomic status among paper mill workers (RR 1.6; 95%CI 1.0 - 2.7) and among pulp mill workers (RR 1.3; 95% CI 1.0 - 1.7).

Tvedt et al. (1991a) Case report of an H_2S-exposed shipyard worker.	ECG showed sinus tachycardia and signs of anterior wall ischemia.
Bhambhani and Singh (1991) 16 young, healthy male volunteers were exposed to 0, 0.5, 2, or 5 ppm H_2S for more than 16 minutes after graded exercise performed to exhaustion.	Heart rate and expired ventilation were unaffected. Oxygen uptake tended to increase and carbon dioxide output tended to decrease. Blood lactate levels were elevated 65% in men after exposure compared to non-exposed controls. Blood lactate concentrations increased significantly during submaximal and maximal exercise for the 5 ppm exposure, however the maximal physical work capacity was not significantly reduced.
Ahlman et al. (1991) Mortality rates were studied among sulfide ore miners (14,782 person years) in north Finland between 1965 and 1985. Deaths in the township of Outokumpu were identified through death certificates and compared with company personnel records. Occupational history, smoking habits and alcohol consumption was obtained from workers or next of kin. The worker death rates were compared to the regional rates.	Compared to the general male Finnish population, a two-fold excess mortality among miners was found mainly due to ischemic heart disease. Of the 44 miners who died from ischemic heart disease, 20 were drillers or chargers exposed to nitroglycerin in dynamite charges but also to several simultaneous stress factors (PAH, noise, vibration, heavy work, accident risk, and working alone). A 2-fold increase risk of ischemic heart diseases was also found in the group of copper miners.
Jappinen and Tola (1990) Cardiovascular mortality study of Finnish men employed in sulphate mills. Death certificates of workers that had been employed continuously for one-year between 1945 and 1961 were followed up until 1981 and compared with national death rates.	From a total of 2268 person-years of data on men exposed to sulphur dioxide, an increased number of cardiovascular deaths (standardized mortality ratio of 123; 95%CI 79 - 184) was observed. These deaths were due principally to an increase in coronary deaths (SMR 145; 95%CI 86 - 229). Among men exposed to hydrogen sulphide and organic sulfides, from a total of 4179 person-years of follow-up, compared to the national death rates, an excess of cardiovascular deaths was observed (SMR 150; 95%CI 105 - 206), due principally to ischemic heart disease (SMR 150; 95%CI 97 - 222). Among men employed more than 5 years and with a follow-up exceeding 15 years, the SMR for cardiovascular diseases was 173 (95% CI 109 - 262) and for ischemic heart disease was 162 (95% CI 88 - 272). Common risk factors could not explain the findings in this cohort.

Table 3 Cardiovascular System Effects: Animal Studies

Study Design	Key Findings
Zhao et al. (2001) The effect of H_2S in regulating cardiovascular function was assessed *in vivo* and *in vitro*. Blood pressure changes were measured in rats exposed to H_2S-containing saline, vascular tension was measured *in vitro*. Biochemical and molecular biology techniques were also used to detect expression of H_2S-generating enzymes.	A transient (29.5 sec) decrease in mean arterial blood pressure was observed (12.5 +\- 2.1 mm Hg; 29.8 +\- 7.6 mm Hg) after intravenous bolus injection of 2.8 and 14 umol H_2S /kg, respectively. Heart rate was not altered. H_2S relaxed rat aortic tissues *in vitro* in a concentration-dependent manner (IC_{50} 125 +\- 14 uM). Cystathionine gamma lyase was detected in endothelium-free rat pulmonary artery, mesenteric artery, tail artery and aorta as well as rat liver. H_2S production from vascular tissues was enhanced by nitric oxide.
Lovati et al. (1996) Sprague-Dawley and streptozotocin diabetic rats were fed on either standard or cholesterol enriched diets (1% cholesterol and 0.5% cholic acid) for 2 weeks. The animals were exposed to 5 or 10 ppm SO_2, 24 hr/day for 14 days in a controlled atmosphere chamber. Triglyceride production, plasma lipids (cholesterol, triglyceride, glucose and free fatty acid concentrations) and insulin levels were determined.	In rats, both on a standard diet and on the cholesterol-enriched diet, SO_2 exposure was associated with a significant dose-dependent increase in plasma triglycerides. This same concentration significantly reduced HDL cholesterol levels. In contrast, exposure of diabetic animals to 10 ppm SO_2 resulted in a fall of plasma and liver triglycerides and a concomitant increase, although not statistically significant, in plasma HDL cholesterol.
Baldelli et al. (1993) Ventilated and unventilated rats were injected intraperitoneally with sodium sulfide to determine its effects on brain function and morphology and physiological parameters such as blood pressure, EEG, arterial blood gases and pH.	Sulfide was found to potently depress blood pressure.
Kohno et al. (1991) Male Wistar rats were exposed to 75 ppm H_2S for 20 to 60 minutes.	The highest level of H_2S was obtained in the heart, followed by kidney, brain, spleen, liver and lung. Serum SGOT was elevated. Delayed P-Q intervals were observed during the 60-min exposure. Cardiac arrhythmia, suggestive of a stimulus transmission disorder was found. Heart rates were 10 - 27% less than controls during exposure and up to 1 hour post-exposure. Histopathological examination showed slight congestion in the lungs.
Seredenko et al. (1991) Rats inhaled an air mixture with hydrogen sulphide containing natural gas. Lipid peroxidation and glycolysis was evaluated.	Lipid peroxidation was inhibited and an increase of blood lactate concentration and lactate/pyruvate ratio was found.
Hayden et al. (1990c) Gravid rat dams were exposed to H_2S (20, 50 and 75 ppm) from day 6 of gestation until day 21 postpartum.	Cholesterol was slightly elevated in maternal liver and brain. Cholesterol was also elevated in the liver and brain of pups exposed to 50 ppm by day 14, but returned to control levels by day 21.

may interfere with T4 to T3 conversion. Perlman et al. (1995) and Cook et al. (1996) have provided strong evidence that a disulfide bond between Cys-98 and Cys-197 in the thyrotropin-releasing hormone receptor is critical for maintaining its high affinity conformation. Dithiothreitol, a disulfide bond reducing agent, reduced specific thyrotropin releasing hormone binding in a dose-dependent manner (Cook et al., 1996).

Rabbits injected with the disulfide reducing agent dithiothreitol, known to penetrate the blood brain barrier, induced a dose-dependent fall of core temperature within minutes (Riedel and Maulik, 1999). Significant core body temperature reductions were measured in rats exposed to 80 ppm or more H_2S for 3 hr/day for 5 consecutive days (Struve et al., 2001). These findings agree with the earlier observation of a dose-dependent reduction in rectal temperature in mice following exposure to H_2S (Hays, 1972; Table A6 in Appendix I). In goats, a stress response, measured as a 48% and 55% increase in plasma cortisol, was induced by a 4-day exposure to 50 and 100 ppm, respectively (Hays, 1972; Table A6 in Appendix I). Corticotropin-releasing hormone release was significantly reduced with exposure to 1 and 10 mM sodium hydrosulfide in an explanted hypothalamic organ system (Russo et al., 2000). At autopsy, an acute exposure produced microscopic 'passive congestion' in the adrenal gland, among other tissues (Chaturvedi et al., 2000).

The dose-dependent delayed delivery time produced by H_2S reported by Hayden et al. (1990b) was supported by earlier findings that 0.15 - 15 uM sodium sulfide inhibited oxytocin-induced rat uterine contractions (Hayden et al., 1989) and more recently by Sidhu et al. (2001). Changes in prostaglandin production, oxytocin or its receptor by sulfide were suggested as possible mechanisms (Hayden et al., 1990b). In an organ bath system, sodium hydrosulfide (nM - mM range) applied to rat uterine strips inhibited spontaneous uterine contractions in a dose-dependent manner (Sidhu et al., 2001). Reductions in the contractility were seen at 10 nM, although not statistically significant from controls until 0.1 mM. These findings are also consistent with the anecdotal observations of exposed animals having difficulty 'pushing' during delivery (Whitelock, 1999; Kostuch, 1999). Reduced milk production, a symptom associated with chronic exposure to H_2S (Rosenberger, 1994) that has been reported by farmers (Church, 1992) also supports the involvement of oxytocin, which is involved in stimulating milk production. As little as 50 ng of infused oxytocin, a short-lived 9-amino acid peptide with a ring structure formed by a disulfide bridge, induces labor. Pretreatment of oxytocin with dithiothreitol impairs receptor binding in adipocytes (Boland and Goren, 1987) however pretreatment of adipocyte oxytocin receptors also impairs oxytocin binding, suggesting that both oxytocin and its receptor are susceptible to reduction by H_2S.

Inhibition of uterine smooth muscle contraction following exposure to H_2S is similar to effects seen in other smooth muscle tissues. Sulphide appears to be produced endogenously and involved in smooth muscle relaxation in the rat ileum, portal vein and thoracic aorta (Hosoki et al., 1997) and in the rat aorta (Zhao et al., 2001). Ileum, portal vein and thoracic aorta produced 20.3, 19.6 and 33.7 nmol H_2S /min/gram of protein. The portal vein (EC50 160 uM), ileum (EC50 180 uM) and thoracic aorta (EC50 >1 mM) were all relaxed by sodium hydrosulfide in a dose-dependent manner. Smooth muscle relaxation was significantly enhanced (approx 13-fold) in a synergistic manner by NO-producing agents (Hosoki et al., 1997), which support prior observations (see Table A6, Appendix I). The effects of H_2S on smooth muscle may be responsible for the increase rates of cholecystitis (gall bladder disease), cholangitis (bile duct disease), and cholelithiasis (gallstones) in oil refinery workers previously documented (Bulatova et al., 1968; see Table A5 in Appendix I).

Several studies have demonstrated dose-dependent inhibition or activation of several key endocrine system signaling molecules. Physiological concentrations of H_2S increased the production of cAMP in neurons and oocytes in culture (Kimura et al., 2000). Most hormones are small molecules, exist in the circulation physiologically at low levels (i.e. nM), and act via cAMP as a second messenger at target cell membranes. Hormone receptors are often G-proteins that have extracellular disulfide bonds essential for receptor conformation and binding. These combined features increase the susceptibility of hormone system proteins to disruption by strong reducing agents such as H_2S.

2.3.1 Limitations and Gaps

Concentration-dependent smooth muscle relaxation, which appears to be augmented by nitric oxide, is consistently observed following H_2S exposure in various tissues. This effect was observed in rats at 20 ppm H_2S (no lower threshold was established) interfering with normal newborn delivery in rats (Hayden et al., 1990b). Zhao et al. (2001) suggested that the mechanism was possibly via opening of potassium ATP channels. However, gap junctions, also known to play a role in passage of action potentials in smooth muscle (Barr, 1996), contain disulfide bonded antiparallel beta sheets (Foote et al., 1998), which are susceptible to disruption by H_2S. Further studies should be undertaken to assess the role of H_2S in smooth muscle function.

Similarly low-level exposures produced dose-dependent reductions in core temperature of mice (Hays, 1972; See Table A6 Appendix I). These studies and the findings of increased sweating and thirst in sewer workers (Watt et al., 1997) raise important questions about the effects of H_2S and other reduced sulfur gas exposure on the hormone cascades involved in thermoregulation and in general stress responses. The widespread distribution of hormone cascades (G-coupled proteins, cAMP) that may be affected by H_2S suggests a need for further research. The prevalence of fatigue resulting from altered thyroid function should also be investigated.

Given the frequent reports of altered lipid and carbohydrate metabolism by H_2S and other sulfur compounds (See Tables A5, A6; Appendix I) such as sulfur dioxide (Lovati et al., 1996), further studies are warranted to clarify the role of sulphur pollutants in endocrine dysfunction such as diabetes.

2.4 GASTROINTESTINAL EFFECTS

Several studies in the past decade (Table 6) have provided further evidence for the relationship between exposure to low concentrations of H_2S and gastrointestinal effects supporting earlier findings (Tables A7, A4.2, Appendix I). Prior to 1990, many studies reported nausea[7] and vomiting following inhalation of H_2S by humans. A number of studies undertaken in the past decade have documented gastrointestinal effects, specifically nausea, following low level H_2S exposure (Table 6). The effects observed at the cellular level are described in Section 2.9.3. (Effects on Mucosal Tissues).

Increased gastrointestinal symptoms have been reported by people living near many types of industrial facilities: an oil battery (Kraut, 2000); a refinery (TNRCC, 1998); pulp mills (Marttila et al., 1995; Haahtela et al, 1992); manure lagoons (Anonymous, 1996); hog farms (Wing and Wolf, 2000); and geothermal electric power plant and industrial wastewater holding ponds (Legator et al., 2001). Studies that have reported corresponding atmospheric H_2S measurements are discussed below.

In communities exposed to pulp mill emissions, a two-fold increase in nausea was found at daily mean TRS concentrations of 11 - 14 μg/m3 (Marttila et al., 1995; Haahtela et al, 1992). As H_2S comprises approximately 2/3 of the TRS in the ambient air at the mills, the H_2S exposures would be in the range of 7 ppb. Increased nausea was reported to be four times more frequent among citizens living near an oil battery, compared to individuals living at a distance from the site (Kraut, 2000). In the vicinity of the battery, 10 one-hour readings were greater than the provincial guideline of 11 ppb, with one peak at 36 ppb. Near manure lagoons, where people experienced nausea and vomiting, all of the sites were said to have some H_2S in the air (Anonymous, 1996). Two of the tests showed mean H_2S levels of more than 100 ppb, with one site measuring 134 ppb. Eight of 32 tests showed mean H_2S concentrations greater than 50 ppb. Air monitoring workers have also experienced nausea and other symptoms after about 5 hours exposure to 90 ppb H_2S downwind from an oil refinery (TNRCC, 1998).

Many anecdotal reports have been located that suggest that low-level H_2S produces a variety of gastrointestinal disturbances (Nikiforuk, 2001; p. 38, 43, 103).

2.4.1 Limitations and Gaps

The recent studies have further illustrated, albeit not completely, the relationship between low level exposure and effects on the gastrointestinal system. A consistent pattern of increased nausea associated with exposure to H_2S in the range of less than 10 ppb to ~130 ppb has been identified. These studies also support previous work showing that H_2S in the ppb range can produce an increased incidence of nausea. The studies showing the lowest levels of effects were by Marttila et al. (1995), Haahtela et al. (1992) and Kraut (2000). The recent study by Kraut (2000) does support the earlier findings of Marttila et al. (1995) and Haahtela et al. (1992) and demonstrates that the incidence of nausea increases with exposure (Marttila et al., 1995). These effects occurred at very low concentrations near 7 ppb (as a daily mean) or 11 ppb H_2S (as a one-hour average). In the vicinity of manure lagoons, where people experienced nausea and vomiting, the atmosphere of all the sites contained some H_2S and 24/32 (75%) of the tests showed mean concentrations less than 50 ppb (Anonymous, 1996).

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[7] As nausea may be triggered by sensory systems in the gastrointestinal tract, the area prostrema of the brain, or other pathways (Lang, 1999), it perhaps should not be considered solely a gastrointestinal effect.

Table 4 **Endocrine System Effects: Human Studies**

Reference / Study Design	Key Findings
Beelen et al. (2000) In a historical cohort study of 7369 workers who worked for at least one year between 1955 - 1992, mortality rates from diabetes mellitus and rheumatoid arthritis among workers at 3 pulp and paper mills in Sweden were compared with Sweden as a whole.	Mortality from diabetes mellitus in the total cohort was close to expected (SMR 1.02). There were increased risks for the job categories of sulphite digestion (SMR 8.54; 95% CI 1.03 - 30.9) and paper and paperboard manufacturing (SMR 5.11; 95% CI 1.06 - 14.9). Increased risks of rheumatoid arthritis mortality were also found in one mill for men (SMR 8.92; 95% CI 1.08 - 32.2) and for the total group of 2 mills (SMR 3.45; 95% CI 1.12 - 8.05).
Chaturvedi et al. (2000) Case report of fatal exposure to H_2S. A male truck driver had accidentally transferred sodium hydrogen sulfide from his tanker truck into a tank containing 4% sulfuric acid and iron sulfate.	Blood sulfide levels were measured at 1.68 µg/mL (about 34 times higher than the sulfide concentration expected in the blood of normal subjects (< 0.05 µg/mL)). Prevalence of pulmonary edema, and congestion in the lungs and kidneys was found on internal exam. Microscopically, passive congestion was evident in the lungs, spleen, kidneys and adrenal glands.
Watt et al. (1997) A clinical follow-up of 26 sewer workers exposed to unusually highly odorous incident during an investigation. Analysis of the sewer gas showed the presence of a mixture of thiols and sulfides, known to be highly odorous but not normally found in sewers.	14/26 workers developed subacute symptoms including sore throat, cough, chest tightness, breathlessness, thirst, sweating, irritability, and loss of libido. The severity of symptoms appeared to be dose-related. One worker had evidence of mild cranial diabetes insipidus.
Wingren et al. (1991) A case-referent study of 4070 pulp and paper mill workers deceased between 1950 and 1987 in Sweden was undertaken. The subjects were identified from the register of deaths and burials in six parishes.	A significantly increased mortality was seen for diabetes mellitus (OR 1.3; 90%CI 1.0 - 1.7) and for secondary tumors of the lung and liver among the pulp and paper mill workers (OR 8.1; 90%CI 2.7 - 24.4).

Table 5 Endocrine System Effects: Animal Studies

Reference / Study Design	Key Findings
Zhao et al. (2001) The effect of H_2S in regulating cardiovascular function was assessed in vivo and in vitro. Blood pressure changes were measured in rats exposed to H_2S-containing saline, vascular tension was measured in vitro. Biochemical and molecular biology techniques were also used to detect expression of H_2S-generating enzymes.	A transient (29.5 sec) decrease in mean arterial blood pressure was observed (12.5 +\- 2.1; 29.8 +\- 7.6 mm Hg) after intravenous bolus injection of 2.8 and 14 umol H_2S /kg, respectively. Heart rate was not altered. H_2S relaxed rat aortic tissues *in vitro* in a concentration-dependent manner (IC_{50} 125 +\- 14 uM). Cystathionine gamma lyase was detected in endothelium-free rat pulmonary artery, mesenteric artery, tail artery and aorta as well as rat liver. H_2S production from vascular tissues was enhanced by nitric oxide.
Sidhu et al. (2001) Using an organ bath system, the effect of 10^{-8} - 10^{-3} M L-cysteine, sodium hydrosulfide (NaHS) and H_2S donors on rat uterine contractility was studied.	L-cysteine and NaHS produced significant dose-dependent decreases in uterine spontaneous contractility.
Russo et al. (2000) The effects of NaHS on corticotropin releasing hormone (CRH) release from rat hypothalamic explants were tested. The explants were prepared in such a manner that preserved the anatomical integrity of the CRH neuron pathway. The hypothalamic halves from the same animal were incubated in the same vial. First a basal rate of CRH release was measured, then NaHS was added to the tissue for a period, and finally potassium chloride was added to assess residual releasing ability. Lactate dehydrogenase, corticosterone, and arginine vasopressin was also measured in the tissue and incubation medium. Hypothalami were also examined by electron microscopy.	NaHS in the range of 0.1 to 10mM did not affect the basal CRH release, however a dose-dependent inhibition of CRH release following KCl treatment was observed, with significant reductions obtained with both 1 and 10 mM NaHS. No microscopic cellular changes appeared to be associated with NaHS dose. The authors noted that interpretation of their data is difficult due to the unresolved problem of blocking in vivo H_2S generation.

Riedel and Maulik (1999) Rabbits were injected with the dithiothreitol (DTT), and its effect on normal and febrile body temperatures induced by lipopolysaccharide (LPS) were monitored.	DTT, which is able to penetrate the blood-brain barrier, induced the full pattern of heat loss responses causing a fall of core temperature, indicative of a lowered thermoregulatory setpoint. The effects were produced within minutes, were independent of ambient temperature and were dose-dependent. Pretreatment with a bolus dose of 5 mg/kg DTT, followed by a continuous infusion of 5 mg/kg/h for 3 h completely prevented LPS-induced fever. A bolus dose of 20 mg/kg DTT, starting 30 min after LPS, immediately reversed the febrile cold defense pattern and lowered body temperature.
Hosoki et al. (1997) Ileum, portal vein and thoracic aorta tissues were examined for expression of cystathionine gamma lyase, cystathionine beta synthase (CBS), H_2S production and relaxation behavior in combination with nitric oxide-producing agents.	CBS was expressed only in the ileum; cystathionine gamma lyase was expressed in all three tissues tested. Ileum, portal vein and thoracic aorta produced 20.3, 19.6 and 33.7 nmol H_2S /min/gram of protein. The portal vein (EC 50 160 uM), ileum (EC50 180uM) and thoracic aorta (EC50 >1 mM) were all relaxed by sodium hydrosulfide in a dose-dependent manner. Smooth muscle relaxation was significantly enhanced (approx 13-fold) in a synergistic manner by NO-producing agents.
Lovati et al. (1996) Male rats and streptozotocin diabetic rats fed on either standard or cholesterol enriched diet were exposed to 5 or 10 ppm SO_2 for 24 hr/day for 14 days. Plasma insulin, triglycerides, cholesterol, and HDL-cholesterol were measured after the exposure.	SO_2 exposure produced a dose-dependent increase in plasma triglycerides in rats, both on a standard diet and a high cholesterol diet. In the diabetic animals, a reduction of plasma and liver triglycerides and a concomitant increase of plasma HDL cholesterol were found. Insulin levels in the diabetic rats increased with increasing SO_2 concentration whereas the rats fed both standard diet and high cholesterol diet decreased with increasing SO_2 concentration.
Hayden et al. (1990b) Sprague-Dawley rats were exposed to 0, 20, 50, or 75 ppm H_2S for 7 hrs/day on gestation days 6 - 21.	A dose-dependent increase in parturition time and difficult delivery was noted in 6/10 animals from all concentrations compared to 1/17 controls. No threshold for the effect on parturition time could be determined.
Hayden et al. (1990c) Rat dams and pups were exposed to 20, 50 and 75 ppm H_2S from day 1 of gestation until day 21 post-partum. Blood chemistry parameters were evaluated.	Blood glucose was significantly elevated in maternal blood on day 21 postpartum at all exposure levels.

Table 6 **Gastrointestinal System Effects: Human Studies**

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Kraut (2000) Environmental exposures to H_2S, SO_2 and volatile organic compounds (VOCs) and symptom severity and frequency were assessed in families that lived around an oil battery in Manitoba.	Elevated SO_2 readings above the air quality objective (0.32 ppm) had been recorded on various occasions. H_2S concentrations measured around the battery showed 10 one-hr readings greater than the provincial guideline of 11 ppb with a peak of 36 ppb. VOC concentrations were comparable to, and in some cases lower than the city of Winnipeg. Compared to individuals not living near the battery, greater than a 2-fold increase in reported symptoms was found for: throat, chest heaviness, nausea, diarrhea, urine symptoms, joint swelling, musculoskeletal symptoms, and 'other' symptoms.
Wing and Wolf (2000) Residents of 3 rural communities (one near an intensive hog operation, one near two intensive cattle operations and a third without livestock operations that use liquid waste management systems) were surveyed to assess health symptom and quality of life indicators.	Residents living near hog operations reported episodes of heartburn (1.94 times), nausea/vomiting (3.46 times), no appetite (3.03 times) and diarrhea (2.96 times), more than the reference community. Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community. Residents living near cattle operations reported episodes of headaches 1.57 times more than the reference community.
Anonymous (1996) Air testing was done near 17 large-scale hog manure lagoons, some as large as a city block, in Renville County by Land Stewardship Project members and staff.	Area residents complained of nausea, headaches, blackout periods, vomiting and other symptoms. During a 2-week period in May, a Jerome Analyzer was used to measure H_2S concentrations near the lagoons as well as up to 1.5 miles away. All of the sites showed some H_2S in the air. Two of the tests showed mean H_2S levels of more than 100 ppb, with one site measuring 134 ppb. Eight of 32 tests showed mean H_2S concentrations of more than 50 ppb.
TNRCC (1998) Six workers were exposed to a mean concentration of 0.09 ppm H_2S for approximately 5 hours in a monitoring van downwind from an oil refinery.	Persistent odors, eye and throat irritation, headache and nausea were observed in the workers.

Marttila et al. (1995) Six consecutive questionnaires were administered to two communities after three pre-defined exposure levels to evaluate the health effects associated with pulp mill emissions.	A 2-fold increased risk of nausea [95% CI 0.53 - 8.77] was associated with the medium and a 4-fold increased risk for the highest exposure group [95% CI 1.08 - 12.05]. The concentration of TRS, as a daily mean concentration, was 2 - 6 µg/m3 for the reference group, 11 - 14 µg/m3 for the medium, and 44 - 82 ug/m3 for the high exposure group.
Anonymous (1995) Investigators of the National Institute for Occupational Safety and Health conducted a health hazard evaluation at a wastewater treatment plant in Independence, Missouri.	Employees reported gastrointestinal disturbances and other complaints while working in the belt pressroom. Measurements of personal breathing zone concentrations of H_2S were: maximum 10-minute ranged from 0.1 ppm to 95 ppm; 8 of the 13 personal breathing zone samples exceeded 10 ppm and 3 exceeded 20 ppm. Maximum 10-min concentrations in general air samples obtained in the belt pressroom range from 46 - 69 ppm whereas outside the belt pressroom, the maximum 10-min concentrations ranged from non-detectable to 0.1 ppm.
Haahtela et al. (1992) Acute health effects were evaluated in 2 communities near a pulp mill, which released emissions containing H_2S as a major component and mesityloxide as a minor component. Questionnaires were administered after a 'high' exposure period and 4 months later after a 'low' exposure period.	Significant increases of nausea were reported during the high exposure period (23% of respondents) as compared to the low exposure period (5%). During the 'high' exposure period, 24-hr average H_2S concentrations were 35 and 43 ug/m3 (25 - 31 ppb), with a 4-hr peak value of 135 ug/m^3 (96 ppb). During the 'low' or reference period, the 24-hr average H_2S concentration was 0.1 - 3.5 ug/m^3.

The people in the above reports were also exposed to other substances, at very low concentrations. This makes it difficult to ascribe the effects of nausea to H_2S per se. However, in virtually all 'real-life' exposure scenarios, H_2S is rarely present as the sole contaminant and yet its presence is the strongest predictor for odour responses to organic wastes (Hobbs et al., 2000).

To further clarify the time-concentration relationships of low level exposures, personal and area sampling with sensitive continuous dataloggers able to detect H_2S in the ppb concentration range combined with time activity diaries and meteorological data (windspeed, direction, temperature, atmospheric stability, etc) are suggested for future studies. Determination of threshold levels may also be valuable in establishing ambient guidelines. Future studies are required to evaluate the gastrointestinal effects of airborne and H_2S dissolved in water, as well as in combination with other substances found in air, water and food. In addition, the role of the olfactory system (see Section 7.11.1) in triggering the gastrointestinal responses should be examined.

2.5 GROWTH AND DEVELOPMENT

It appears that human studies evaluating the effects of H_2S exposure on growth and development were not conducted during the past decade, and only two animal studies were located (Table 7).

Dorman et al. (2000) reported that both male and female rats exposed to 80 ppm had only a 5 - 6% weight reduction throughout the exposure period. Hayden et al. (1990b) found a trend, although not statistically significant, toward a dose-dependent reduced weight gain in rats. Prior to 1990, there were several studies that reported an overall pattern of reduced weight gain under chronic exposure conditions (Table A8 in Appendix I). Reduced feeding rates in exposed rats were also described by Hayden et al. (1990b), in agreement with previous studies (Table A8, Appendix I). In most cases, the food consumption tended to recover over time. Reduced milk production (Kostuch, 1992; Bocock, 1992) and consistent with the controlled studies, feeding rates (Whitelock, 1992; McGlynn, 1992; Kostuch, 1992; Harris, 1992), have also been reported frequently by farmers.

No significant differences in development were observed between controls and exposed animals in the study undertaken by Dorman et al. (2000). Hayden et al. (1990b) found that animals exposed to 20 ppm H_2S from day 6 of gestation until day 21 postpartum had delayed ear detachment, and animals exposed to both 20 and 50 ppm had delayed hair development. Dorman et al. (2000) referred to earlier occasional reports of structural malformations in newborn animals such as wavy ribs, kinked tails, tail agenesis (failure of development), frontal bone holes, and hypognathia (lower jaw longer than the upper). Also, epidermolysis bullosa-like skin lesions characterized by non-inflammatory separation of the epidermis from the underlying dermis in 50% of one litter were described (Dorman et al., 2000). The above findings indicate that subtle effects may be occurring on collagen development, and provides support for the work of Johnson et al. (1992b) (See Section 2.9.2.).

The above findings are compatible with anecdotal reports of exposed animals having reduced weight gains (Church, 1992; Kostuch, 1992; Whitelock, 1992; McGlynn, 1992; Harris, 1992), unspecified deformities in offspring of sows bred during a blowout (Harris, 1992), deformities in the front legs of cattle (Church, 1992, McGlynn, 1992), and difficulty or inability to stand up or walk properly (Whitelock, 1999; Johnston, 1999).

2.5.1 Limitations and Gaps

Reduced weight gains, frequently reported by farmers and attending veterinarians have been associated with H_2S exposure. However, controlled experimental studies remain controversial and necessitate further study. The few recent findings imply that reduced weight gain of chronically exposed animals (i.e. rats) may occur, however concentrations range considerably (e.g. 7- 80 ppm), and the effects are considered to be subtle. Thus the question of whether the reduction of weight gain can be attributed to H_2S remains unanswered. This supports further research involving long-term monitoring of exposed farm animals to evaluate the exposure-response relationship. These data could then be compared with those of rats and mice. The monitoring should be undertaken with sensitive datalogging instruments for H_2S, animal weight gain, feed, water, etc. as well as weather conditions (i.e. wind direction, speed, atmospheric dispersion). Since cattle appear to be susceptible to increased sulfur (sulfide and sulfate) in their feed and drinking water (Gould, 1998; Oude Ellerink, 2000), these parameters must also be taken into consideration.

Growth is influenced by many factors, including provision of adequate nutritional food and functional cellular processes[8]. As identified in other sections of this report, H_2S can affect many aspects of cellular metabolism and processes that are highly inter-dependent. For example, growth hormone is present in the circulation at concentrations of < 10 ng/mL (Wood, 2001), and is a major regulator of growth in soft tissue, cartilage and bone, including linear bone growth, lactation, activation of macrophages, and insulin-like and diabetogenic effects. It is a pulsatile secretion that is influenced by neurogenic, metabolic and hormonal factors. Growth hormone action may therefore be affected by H_2S via several mechanisms. Further, the structural similarity between growth hormone and prolactin, which contains zinc in the receptor binding configuration (Cunningham et al., 1990), suggests that parallel effects of growth retardation and reduced milk production following H_2S exposure may be related biochemically and/or physiologically.

Many of the developmental effects reported, although sporadic, suggest alterations in connective tissue development. Inhibition of the copper enzyme[9], lysyl oxidase or disruption of extracellular matrix proteins containing disulfide bonds such as collagen, fibronectin, fibronectin receptor, link protein, laminin or others may explain the structural developmental alterations (See also Section 2.10.2.4 - Neural Cell Growth). Inhibition of the zinc metalloenzyme, alkaline

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[8] Six sulfur-containing cofactors have been identified in vertebrates: coenzyme A, S-adenosylmethionine, thiamin pyrophosphate, biotin, molybdopterin, and lipoic acid.

[9] Recall that the solubility product constant for CuS is very low (reported values of 10^-36 to 10^-38) and for Cu_2S is lower (10^-48). This indicates that, when both ions are present, very little of each are needed for complex formation; also that the complex does not readily dissociate once formed.

Table 7 **Growth and Developmental Effects: Human and Animal Studies**

Reference / Study Design	Key Findings
Dorman et al. (2000) Rats were exposed to H_2S at various concentrations (0, 10, 30, 80 ppm; 6 h/day; 7 days/wk) for 2 weeks prior to breeding, for a 2-wk mating period, and then from gestation day 0 (gestation day 0 = evidence of copulation) through day 19. Exposure of dams and their pups resumed between postnatal day 5 and 18. Adult male rats were exposed for 70 consecutive days. The animals were subjected to various neurological tests (motor activity, passive avoidance, functional observational battery, acoustic startle, neuropathology), at various times during the exposure period. At the end of exposure period, the animals were sacrificed and their tissues examined.	There were no deaths and no adverse physical signs during the study, nor any statistically significant effects on reproductive performance. Exposure to H_2S did not affect pup growth, development, or performance on any of the behavioral tests. Body weights of female and male rats exposed to 80 ppm were decreased 5-6% during exposure.
Hayden et al. (1990b) Gravid rat dams were exposed to H_2S (20, 50 and 75 ppm) from day 6 of gestation until day 21 postpartum. Weights were measured at gestation days 1, 5, 9, 13, 17, 21, and post-partum days 1, 7, 14, and 21.	Maternal body weight gains (58% increase) were similar for both the control and exposed dams. However, a trend toward a dose-dependent reduced weight gain, although not statistically significant, was found. Food intake was reduced during the first 4 days of exposure to 50 ppm H_2S and during the first 8 days of exposure to 75 ppm H_2S, however recovered after several days. Brain and liver weights did not differ between controls and the various treatment groups. Gestation length, viability, litter size, or male to female ratio of newborn pups were not altered. Nor was the time to incisor eruption, eyelid opening or surface righting altered. However, animals exposed to 20 ppm H_2S had delayed pinna detachment and animals exposed to both 20 and 50 ppm had delayed hair development, the lower concentration giving the longer delay. No differences were found between exposed and control pup liver, brain or total body weights. No differences were found between exposed and control rat pups or dams for total liver protein or DNA accumulation.

phosphatase[10] by sulfide, may also play a role. Alkaline phosphatase is able to bind extracellular matrix proteins such as collagen and may be a mechanism for the attachment of osteoblasts to cartilage, promoting calcification (Moss, 1992). In addition to the findings outlined in Section 2.9 (Effects on Mucosal Tissues), the few studies during the past decade raise questions about the effects of H_2S on growth and development and warrant additional research be undertaken to assess the role of H_2S exposure on connective tissue development especially *in utero*.

2.6 HEMATOLOGICAL EFFECTS

Few studies during the past decade examined the effects of H_2S on specific hematology parameters. Several of these reported some effects (Tables 8 and 9). Prior to 1990, however, many investigators reported a variety of hematologic alterations associated with H_2S exposure (Tables A9 and A10 in Appendix I).

2.6.1 Erythrocyte and Leukocyte Alterations

Occasionally, in cases of workers or animals acutely exposed to H_2S, white blood cell counts were transiently elevated to approximately 2-4 times normal values (Gregorakos et al., 1995; Snyder et al., 1995; Peters, 1981; van Aalst et al., 2000). Case reports of acutely exposed steers also have mild neutrophilia (Hooser et al., 2000) supporting earlier reports (Table A10, Appendix I) of increased leukocytes in calves exposed to 20 ppm H_2S for 7 days continuously (Nordstrom, 1975), rats exposed to 7 ppm daily for 6hr/day for 4 months (Elebekova et al., 1976) or mice continuously exposed to 20 ppm for 90 days (Sandage, 1961).

Under chronic exposure conditions, landfill workers showed slight but significant increases in leukocytes, neutrophils, lymphocytes, CD8+ lymphocytes and complement C3 compared to controls (Sadowska et al., 1999).

Although leukocytosis is associated with many conditions, it is well established that adrenergic agonists produce transient leukocytosis and that the transient nature of the elevation suggests the cells are derived from the marginating pool[11]. Given that the beta-adrenergic receptor is apparently activated by cleavage of its disulfide bonds (Pedersen and Ross, 1985; Savarese and Fraser, 1992), this could explain the transient leukocytosis found after acute exposures.

Prior studies have shown various hematological effects (Table A9; Appendix I). Compared to the reference community, anemia was reported 7 or 10 times more frequently among citizens exposed to geothermal emissions or wastewater treatment pond emissions, respectively (Legator et al., 2001). In ruminants, anemia is listed as a symptom of chronic exposure to lower

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[10] The solubility product constant for ZnS is reported as 10^-21.

[11] The marginating pool of leukocytes are those that are within the blood vessels but are intimately associated with the endothelium, resisting the shearing forces of flowing blood.

(concentration range not specified) H_2S concentrations (Rosenberger, 1994). Prior studies have shown effects on erythrocyte formation (Tables A9, A10, Appendix I).

In acutely exposed workers, several studies report increased carboxyhemoglobin (Gregorakas et al., 1995; Pach et al., 1996), sulfhemoglobin (Sandage, 1961; Peters, 1981; Gregorakas et al., 1995) or methemoglobin (Schults et al., 1970; Pach et al., 1996) or some combination thereof. Increased sulfhemoglobin and cholehemoglobin, and shape reversion of stomatocytes to discocytes, occurred following exposure *in vitro* to 5 mM H_2S (Moxness et al, 1996). As the absorption spectrum of each of the hemoglobin types overlap to varying degrees, separation of one from the other is not straight forward, even with the algorithms often used in modern analytical instruments (Zoppi et al., 1996; Demedts et al., 1997). These analytical interferences make quantification uncertain and interpretation difficult for exposure to multiple reduced sulfur pollutants and/or carbon monoxide.

It is known that methemoglobin, which cannot reversibly bind with oxygen, is a continually produced oxidation product of hemoglobin. The values are normally very low in the blood due to the cellular reducing enzymes greater rate of activity. However, in erythrocytes, 90% of the ATP produced comes from the glycolysis, which not only is required to maintain hemoglobin function, membrane integrity and deformability, erythrocyte volume, but is also used to generate adequate amounts of reduced pyridine nucleotides to keep the iron in hemoglobin in a reduced state. Of the 10 enzymes in the glycolysis pathway, 7 are inhibited to varying degrees by persulfide generators or mercaptans (Valentine, 1984), and 5 of those 7 enzymes require magnesium as a cofactor (Voet, 1999, p 384). Due to the high affinity of magnesium to sulfide, the likely mode of inhibition is due to sulfide binding to the magnesium cofactor, neutralizing magnesium's ability to shield the negative charges of ATP's phosphate oxygen atoms. Also, several antioxidant enzymes are inhibited by sulfide (catalase, SOD, and glutathione reductase). The combined effects would be a decreased production of reducing equivalents, a decreased production of ATP, and inhibition of anti-oxidant enzymes for limiting iron-mediated oxygen radical species production, shortening erythrocyte lifespan in the bloodstream.

Sulfhemoglobin is believed to be formed from the addition of H_2S to a double-bonded pyrrole carbon at the periphery of the porphyrin ring in hemoglobin and cannot be reconverted to functional hemoglobin by normal erythrocyte mechanisms or by any known drugs (Liu, 1977; p 321). As porphyrin rings are common structures in various oxidative enzymes[12], H_2S may directly affect similar structures in other proteins increasing their susceptibility to irreversible conversion to non-functional status.

2.6.2 Iron Metabolism Alterations

Church (1992) reported that 3/6 cows exposed to sour gas plant or blowout emissions had high iron levels and 5/6 had low unsaturated iron binding capacity compared to the Western College of Veterinary Medicine reference values. Church's findings are consistent with earlier reports

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[12] Vertebrate heme proteins include: hemoglobin, myoglobin, cytochromes a, b and c, P450, cytochrome c oxidase, catalase, nitric oxide synthase, and peroxidases including myeloperoxidase, eosinophil peroxidase, lactoperoxidase, thyroid peroxidase, prostaglandin H synthase, and peroxidasin.

(Table A9, Appendix I) of altered iron parameters in humans (Benini & Colamussi, 1969; Klingberg et al., 1988) and animals (Kluczek & Kluczek; 1985).

The results suggest that H_2S and mercaptans can interact with the hematological system at several levels. Some of the most clearly established effects are inhibition of several enzymes in the heme synthesis pathway (Savolainen and Tenhunen, 1982; Jappinen and Tenhunen, 1990; Tenhunen et al., 1983; Tenhunen and Savolainen, 1987; Klingberg et al., 1988). Tables 8 and Table A9 in Appendix I summarizes the dose-or exposure-response relationships for each study. Two cysteine residues, two zinc atoms and two histidine residues are involved in the active site of D-ALA synthase (Jaffe, 1995; Tsukamoto et al., 1979), a configuration that is susceptible to binding and inhibition by sulfide.

To compensate for the effects of reduced heme synthesis, the body's normal response is to release into the bloodstream slightly immature red cells from the bone marrow (reticulocytes), as was observed in animal studies. However, the observation of a 'scanty' marrow (Tenhunen et al., 1983) suggests additional effects. Erythropoietin, the hormone required for normal erythropoiesis, contains one disulfide bond formed by C7 and C161, which is part of the hormone's active site (Kung et al., 2000). This hormone, normally present at low picomolar concentrations in the blood, may be susceptible to cleavage by sulfide. The alterations reported by Tenhunen et al. (1983) were observed in workers exposed to low levels of pollutants (H_2S 8-hour time-weighted average of 0.05 to 5.2 ppm), methylmercaptan (0.07 to 2.0 ppm) and dimethylsulfide (0.03 - 3.2 ppm). While there are no reports of clinical anemia *per se*, report of 'scanty' bone marrow (Tenhunen et al., 1983) indicates that hematopoietic cell formation has been affected.

Recent work of Cassanelli and Moulis (2001) may explain the findings of elevated serum iron and transferrin and low ferritin in occupationally exposed workers (Klingberg et al., 1988). This combination of results is not known to occur spontaneously or as a result of known disease. Cassanelli and Moulis (2001) have shown that under anaerobic conditions in the presence of limited amounts of sulfide (0.5 - 2.0 mM), a continuous rate release of iron from ferritin was observed, confirming prior findings that the iron storage protein apoferritin (protein shell minus iron), releases iron when confronted with reducing agents (Bauer, 1984). The chronic low-level H_2S exposure, releasing iron from ferritin, would explain the elevated serum iron values concurrent with low ferritin values.

Given the association between iron and mitochondrial energy metabolism (Hentze and Kuhn, 1996), alterations in plasma iron parameters found by Klingberg et al. (1988) may be due to the disruption of Fe-S clusters by H_2S and mercaptans (Beinert et al., 1997), inhibition of cytochrome c oxidase (Gattermann et al., 1997), disruption of iron transport proteins, or some combination of the above.

Fe-S clusters are present in heme synthase (also called ferrochelatase), the terminal step in the heme biosynthetic pathway (Dailey et al., 1994), and in iron regulatory protein-1 (IRP-1), also known as aconitase (the second enzyme in the citric acid cycle), which regulates vertebrate iron metabolism (Paraskeva and Hentze, 1996). In ferrochelatase, the Fe-S clusters have a crucial role in maintaining protein structure such that when the cluster is disassembled, enzyme activity is lost (cited in Sellers, 1998). The iron regulatory protein function is modulated by assembly and disassembly of its iron-sulphur cluster (Beinert et al., 1997). It has been demonstrated that in the presence of excess sulfide, iron-sulphur clusters disintegrate (Beinert et al, 1997). It has also been shown that hypoxia induces a loss of IRP-1 binding activity, suggesting that reactive oxygen species destabilize iron-sulphur clusters in cells (Haile, 1999), and that superoxide can oxidize iron-sulphur proteins to release iron (Flint et al., 1993). Thus, sulfide inhibition of cytochrome c oxidase via its ability to produce hypoxia, generate oxygen and sulfide radicals thereby leading to an $Fe3^+$ accumulation in the mitochondria and reducing the availability of ferrous iron for ferrochelatase, are possible pathways to heme synthesis disruption.

2.6.3 Effects on Hemostasis

In the past decade, only one study of chronic low-level exposure examined the effect of H_2S on hemostasis (Legator et al., 2001). It was suggested in early literature that H_2S exposure might affect hemostasis (Table A9; Appendix I). While altered hemostasis does not appear to be sustained in reports of H_2S exposure, several reports, most prominent in acute exposures and occasionally in chronic exposures, do suggest problems with vascular integrity and control.

Diffuse hemorrhages of the gastric mucosa were reported in 5 patients who died following acute exposure to H_2S (Gregorakos et al., 1995). A worker exposed to H_2S in a toilet facility who died had hemorrhagic bronchitis (Parra et al., 1991). Severe bilateral subconjunctival hemorrhages, diffuse petechiae found with bronchoscopy and facial petechiae were observed in workers acutely exposed to H_2S (van Aalst et al., 2000). Bleeding was reported in a case of neonatal encephalopathy attributed to chronic H_2S exposure (Walker et al., 1997). Similar reports of altered hemostasis following acute H_2S exposure were reported prior to 1990 (Table A10; Appendix 1).

In communities exposed to chronic low levels of H_2S, self-reports are suggestive of problems in hemostasis. Compared to reference communities, increased reports of clotting disorders, bruising easily, abnormal blood counts and anemia were reported (Legator et al., 2001). In the geothermal and wastewater holding pond communities respectively, 14 and 18% of exposed respondents reported 'bruising easily', whereas fewer reported anemia (7 and 10%), abnormal blood count (5 and 6%) or clotting disorder (3 and 4%). In the reference community, the symptom-reporting rates were 0, 1, 2, and 2% for each category, respectively.

Recently, Rosenberger (1994) notes that ruminants exposed to high concentrations of H_2S show clinical symptoms, among others given, of reduced blood coagulation and hemorrhages in the upper parts of the respiratory tract, whereas subcutaneous bleeding is a symptom of chronic exposure to lower concentrations. This is consistent with an earlier review also has noted changes in coagulation among animals exposed to manure gases (Hogsved, 1968) and with earlier reports (Table A10; Appendix I).

2.6.4 Gaps and Limitations

Despite early studies suggesting effects on the hematological or hemostasis systems, few studies in the past decade have examined the influence of H_2S on these systems. Yet, recent reference textbooks suggest chronic effects in ruminants (Rosenberger, 1994) and self-reported symptoms of anemia or symptoms suggestive of hemostasis disorders among people living in a geothermal area and near wastewater lagoons (Legator et al., 2001) imply that additional studies should be undertaken.

Few studies had information from which dose-response relationships could be derived. Measurements of H_2S concentrations as well as other gases (mercaptans, carbon monoxide, sulfur dioxide), coinciding with effects of interest should be done in populations working in pulp and paper plants, living near landfills, near wastewater treatment ponds or large agricultural operations.

Additional *in vivo* and *in vitro* studies should be undertaken to evaluate the effects of H_2S and methyl mercaptan on proteins and enzymes containing heme, iron-sulfur clusters, ferritin and possible associated secondary effects such as reactive oxygen species generation.

2.7 HEPATIC EFFECTS

Only a few studies during the past decade examined the relationship between hepatic function and H_2S exposure (Table 11). Studies undertaken prior to 1990 are summarized in Appendix I Table A12.

Release of soluble cytosolic enzymes into the circulation following acute exposures has been reported in cattle (Church, 1992; Edwards, 1992; Florence, 1996; Hooser, 2000) and in humans (van Aalst et al., 2000) supporting earlier findings (Burnett et al., 1977; (Table A12 in Appendix I). The enzymes reportedly increased in plasma include: lactate dehydrogenase (LDH), aspartate aminotransferase (AST), creatine phosphokinase (CPK), and alanine aminotransferase (ALT). In rats exposed to 20 - 80 ppm H_2S, increased plasma enzymes are not seen (Hayden et al. 1990a) supporting earlier findings (CIIT, 1983; see Table A12 in Appendix I). Alterations in lipid metabolism affecting cholesterol (Hayden et al., 1990b) have been reported in rats.

An anecdotal report described an unusual observation of an enlarged gall bladder (about 3X normal) from rabbits raised for food and fur on a farm (Ludwig, 1997). This was associated with emissions from a facility 0.5 miles away, intermittently releasing sour gas. Earlier studies reported alterations in bile physiology in animals and workers (Table A12, Appendix I) (Sava et al., 1980; Sava and Giraldi, 1981; Bulatova et al.; 1968; NIOSH, 1977) following H_2S exposure. These findings may be accounted for by inhibition of bile transport by H_2S, as thiol-selective reagents inhibit sodium-dependent bile acid transport and inactivate bile acid uptake in ileal brush-border vesicles (Hallén et al., 2000) or by alterations in smooth muscle function as discussed in Section 6.3.

Prior to 1990, several studies of methyl mercaptan exposure (Table A12, Appendix I) suggested that the liver may be affected by low-level exposure (Tansy et al., 1981; Sandage, 1961).

Table 8 Hematological System Effects: Human Studies

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios [OR] greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
van Aalst et al. (2000) Case reports of two men (48 and 40 years old) acutely exposed to H_2S.	White blood cell counts were 11.5 and 24.5 X 10^3 /mm3. Bronchoscopy performed at 24 hours showed significant edema and diffuse petechiae and patchy mucosal erosions as far as the bronchoscope could be passed.
Sadowska et al. (1999) Immunological parameters were assessed in landfill workers in Belgium by multiparameter flow cytometry.	Compared to controls, landfill workers showed slight but significant increases in leukocytes, neutrophils, lymphocytes, CD8+ lymphocytes and complement C3.
Walker et al. (1997) A case report of neonatal encephalopathy attributed to chronic H_2S exposure.	Bleeding was reported.
Bhambhani et al. (1997) Inhalation of 10 ppm H_2S by volunteers during two 30-minute sessions of submaximal exercise.	Percent hemoglobin saturation was unchanged.
Pach et al. (1996) Report of investigation of health of inhabitants living near a large refuse dump.	Elevated methemoglobin levels were found in 8 patients. Increased blood lactate was found in 14 people and elevated levels of carboxyhemoglobin were found in 8 people. Gases identified from the dump were carbon dioxide, methane, carbon monoxide, hydrogen sulphide, methane homologues and aromatic hydrocarbons.
Snyder et al. (1995) Case report of incident where 37 workers were exposed to H_2S from a pit dug in a coastal wetland.	One of the workers that developed persistent neurological deficits, had carboxyhemoglobin of 14.9% and a WBC of 25,000/uL.
Gregorakos et al. (1995) Report of 8 cases of acute hydrogen sulphide exposure.	All four patients who survived had elevated white blood cells counts (> 15,000/mm3) on admission to hospital. Carboxyhemoglobin and methemoglobin levels were slightly elevated (5.5% - 7.2%; 0.5 - 2.2%, respectively).
Bhambhani et al. (1995) Inhalation of 5 ppm H_2S by volunteers during submaximal exercise.	Percent hemoglobin saturation was unchanged.
Jappinen and Tenhunen (1990) 21 cases of hydrogen sulphide exposure (~1 minute - 3.5 hrs; average duration 4-5 minutes) at sulphate pulp mills in Finland and at a Finnish oil refinery, immediately following exposure to hydrogen sulphide (most likely in the range of 20 - 200 ppm) are described.	In six cases where blood samples were collected in less than 2 hours after poisoning, the mean of the initial blood sulphide concentrations was 75ug/L (range 30 - 130 ug/l). The delta-aminolevulinic acid synthase (ALA-S) activity was lower in subjects than in controls and remained decreased for one week after the incident. The decrease was most prominent in subjects with initially high blood sulfide concentrations. For 3 subjects, the activity was decreased for one month following the incident. No major changes were observed in the heme synthase activity in exposed patients. The erythrocyte protoporphyrin concentration was slightly below the controls one day and also one week after exposure.

Table 9 Hematological System Effects: Animal Studies

Reference / Study Design	Key Findings
Hooser et al. (2000) Two steers that were near death from exposure to manure gases generated from agitation of manure under a slatted floor, were brought to the Purdue Animal Disease Diagnostic Laboratory for clinical evaluation, euthanasia and necropsy.	The animals were recumbent and unresponsive to visual and auditory stimuli. A mild neutrophilia was reported (11.59 and 8.33 X 10^3 cells/uL; normal 1.0 - 4.0 X 10^3 cells/uL). No evidence of viral or bacterial infection was found. Necropsy examination showed no significant gross lesions. Microscopic examination of the brains showed massive, diffuse cerebral cortical laminar necrosis and edema. Portions of the outer lamina contained hypereosinophilic and shrunken neurons. The subcortical white matter was vacuolated in some areas.
Khan et al. (1998) Rats were exposed to 0, 10, and 100 ppm H_2S for 8 hours per day for 5 days/wk for 5 weeks. At the end of the exposure regimen, superoxide dismutase [SOD] activity was determined in the red blood cells, and lung, brain and liver mitochondria.	No significant changes were observed in red blood cells, however there was a slight reduction trend (not statistically significant) in SOD enzyme activity that corresponded with increased exposure.
Church (1992) Case reports of cattle exposed to sour gas emissions from the Lodgepole blowout.	3/6 cows had higher iron levels and 5/6 had low unsaturated iron binding capacity than the reference values.

Table 10 Hematological System Effects: *In vitro* Studies

Reference / Study Design	Key Findings
Searcy and Lee (1998) Isolated erythrocytes were incubated with and without glucose under aerobic and anaerobic conditions.	In vitro, human erythrocytes reduced elemental sulphur in the presence of glucose to form H_2S at a rate of 170 umol $(L\ cells)^{-1}\ min^{-1}$, suggesting that H_2S may act as an electron carrier.
Mariggio et al. (1998) Apoptosis was studied in neutrophils treated with 1 mM sodium sulfide.	Apoptotic fate was enhanced by 1 mM sulfide by increasing the number of cells containing pyknotic nuclei, internucleosomal cleavage, and intensity of tubulin staining. The effect was partially reversible by ionomycin supporting the modulating role of cytosolic calcium.
Moxness et al. (1996) Human erythrocytes and selective oxidants were used to study the role of various hemoglobin oxidation products in lipid reorganization and extraction.	H_2S treatment of erythrocytes produced a 3 - 5-fold increase in cytosolic lipid. Also, partial conversion of oxygenated hemoglobin to sulfhemoglobin and cholehemoglobin, shape reversion of stomatocytes to discocytes occurred following 5 mM H_2S exposure. A positive correlation between hemoglobin oxidation products and cytosolic extraction of membrane components was found. This correlation plus the finding of co-elution of lipid and hemoglobin on both gel filtration and ion exchange chromatography columns, suggested to the authors that hemoglobin derivatives solubilize phosphatidylserine phospholipids.
Searcy (1995, 1996) In vitro studies on the substrate specificity and kinetics of superoxide dismutase [SOD] activity.	SOD can catalyze sulfide (HS-) oxidation according to the reaction: $HS^- + 2O_2^- + 3\ H^+ \text{->} S_o + 2\ H_2O_2$ The superoxide scavenging ability was enhanced 2-fold upon addition of HS-, with an estimated Km ~ 8 - 120 micromolar HS-. Binding of HS- to SOD was rapid ($k>10^7\ M^{-1}\ s^{-1}$) and occurred at the catalytic copper center of SOD, indicating that HS- can be a genuine enzyme substrate. The rate of HS- oxidation by SOD is slower than that provided by other cellular oxidation mechanisms, such as mitochondrial oxidation, hemoglobin, and concluded that the reaction is unlikely to be of physiological significance. However, Searcy (1996) also found that the rate of HS-oxidation started slowly, but gradually accelerated so that after 2 hours, it became several hundred times faster than the initial rate. This observation was attributed to a non-enzymatic oxidative chain reaction or cycle of reactions that is not quite self-sustaining but requires enzyme for initiation and continued reaction. This 'augmented' oxidation has been reported with other oxidases and is suggested to be of concern in oxidative stress.

2.7.1 Gaps and Limitations

A minimum of exposure-response data is available from these studies. Release of cytosolic enzymes occurs predominately under high H_2S exposure scenarios, however, the precise H_2S concentration at which this occurs is usually not reported.

The elevated cytosolic enzymes found after acute exposure are likely from the liver, however, as observed by Gregorakas et al. (1995) and Tanaka et al. (1999), may also originate from other organs, such as cardiac tissue[13]. As such, additional studies on acutely exposed animals or people should attempt to identify the specific isozymes found in the plasma following exposure. This could possibly help to discriminate between reversible and non-reversible cellular damage; generally cellular damage in the liver is considered reversible, whereas, cellular damage to organs with highly differentiated cells and reduced regenerative capability such as the myocardium, is considered non-reversible.

Since there is suggestive evidence that low concentrations of methyl mercaptan may affect liver function, additional studies should be undertaken to evaluate whether there is a combined effect of H_2S and mercaptans.

2.8 IMMUNE SYSTEM EFFECTS

During the past decade, few studies were located that evaluated the effects of H_2S on the immune system (Table 12). Since many immune proteins such as immunoglobulins, complement, T-cell receptors, major histocompatibility proteins, and immunoregulatory molecules such as cytokines, possess multiple disulfide bonds, they may be susceptible to disulfide bond disruption by H_2S.

Legator et al. (2001) found increased rates immune symptoms (specifics were not given) among citizens living in a geothermal area and near industrial wastewater treatment ponds. Compared to controls, landfill workers showed slight but significantly increased leukocytes, neutrophils, lymphocytes, CD8+ lymphocytes and complement C3 (Sadowska et al., 1999).

Granlund-Edstedt et al. (1991) reported that 1 mM sodium sulfide had low capacity to split interchain disulfide bonds of IgG, C1s, C1r, C1q, C4, C5, but no detectable effect on C2, factor B or properdin. An effect on the complement component C3bi was observed. Sodium sulfide split the C-terminal region of the alpha chain from C3bi. This region binds to the complement receptor of the neutrophil in the opsonization process[14], a key immune defense function against capsulated bacteria. Granlund-Edstedt et al. (1993) also showed that 2 mM sodium sulfide cleaves the beta-chain of C3b, C3bi and the C-terminal part of the alpha-chain of C3bi from opsonized bacteria.

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[13] Transaminase acitvities in human tissues, relative to serum (base as one) for AST and ALT, respectively are as follows: heart 7800, 450; liver 7100, 2850; skeletal muscle 5000, 300; kidney 4500, 1200 (Kachmar and Moss, 1976; p. 675).

[14] Opsonization is a process of coating bacteria or other particles with substances present in plasma (antibody or complement) rendering them more 'palatable' for phagocytic cells.

Table 11 Hepatic System Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Hooser et al. (2000) A case report of two H_2S -exposed cows.	Increased serum aspartate aminotransferase (AST) in both animals was found (704 and 1522 U/liter; normal range 58 - 100 U/liter)
van Aalst et al. (2000) Case report of two acutely exposed workers	Increased serum aspartate aminotransferase and alanine aminotransferase was found in one worker.
Florence (1996) The mean activities of enzymes analyzed from blood samples collected from cattle in 10 herds in the Lodgepole area were compared with samples collected 1 year later.	Increased plasma aspartate aminotransferase levels were found in 8/10 animal data sets.
Church (1992) Blood chemistries were measured on 6 cattle exposed to one or more blowouts and/or been downwind of a sour gas plant, chosen by their owners as being typical of the problem.	Increased LDH was found in 6/6 animals; total protein was increased in 2/6 animals; increased CPK was found in 1/6; increased ALT (SGPT) was found in 1/6; and decreased SGOT was found in 1/6 animals.
Edwards (1992) 124 blood samples from cattle exposed to a blowout in Rankin County, Mississippi were analyzed.	LDH and SGOT activities were, on average, elevated in the grouped data.
Hayden et al. (1990a) Sprague-Dawley rat dams were exposed to 20, 50, 75 ppm H_2S for 7 hr/day from gestation day 1 through postnatal day 21.	No changes in serum protein, LDH, SGOT, or alkaline phosphatase were found.
Hayden et al. (1990b) Sprague-Dawley rat dams were exposed to 20, 50, 75 ppm H_2S for 7 hr/day from gestation day 1 through postnatal day 21.	Maternal liver cholesterol levels were increased.

Neutrophil chemotaxis and degranulation was not affected significantly by 1 - 2 mM sodium sulfide (Persson et al., 1993). However in contrast, calcium-dependent cytoskeleton activities such as chemotaxis and azurophilic granule release, was depressed in gingival sulcus neutrophils when exposed to 2 mM sodium sulfide (Mariggiao et al., 1997).

In CBA mice maintained for 4 months near the Astrakhan Gas Complex in Russia, T-cell immunity was more sensitive to the air pollutants than B-cells and mononuclear phagocytes (Bochanovskii et al., 1995). Reductions of thymic and splenic mass and mature T-cell numbers in the spleen were observed. These findings are consistent with those in cattle made by Stair et al. (1996).

Enzymes involved in cellular defense such as myeloperoxidase (MPO) or alkaline phosphatase may also be susceptible to the effects of H_2S. MPO, responsible for intracellular bacterial killing, contains a haem prosthetic group with a histidine proximal ligand, similar to other peroxidases (Isaac and Dawson, 1999), several of which have been reportedly inhibited by H_2S (cited in Beauchamp, 1984). MPO is joined together by three disulfide bonds (one between and two within the subunits) (Andersson et al., 1998).
Thirty percent of the MPO activity and 90 - 95% inhibition of the respiratory burst and superoxide production in bovine neutrophils exposed to 0.5 - 1 mM sulfide was observed (Khan et al., 1998b). When pulmonary macrophages were stimulated with zymosan *in vitro*, a statistically significant reduction in the respiratory rate was seen for macrophages exposed to 30 uM sulfide and 30 uM sulfite. Also, 30 uM sulfide inhibited basal respiratory rates compared to untreated control pulmonary alveolar macrophages (Khan et al., 1991). These findings support earlier studies (Solov'eva, 1970; Robinson (1979, 1980, 1982; Claesson et al., 1989; Table A13, Appendix I).

Overall, these findings appear to be in agreement with observations by farmers (CASA, 1999; McGlynn, 1992), veterinarians (CASA, 1999; Church, 1992; Round, 1992) and community reports (Legator, 2001; Jaakkola et al., 1999; Partti-Pellinen et al., 1996; Jaakkola et al., 1991) describing altered immune responses, increased infections in H_2S-exposed animals, and increased symptoms and childhood respiratory infections in exposed human populations. Studies undertaken prior to 1990 support these observations (Fridyland, 1959; Setko et al., 1989; Kluczek & Kluczek, 1985; Rogers & Ferin, 1981; Table A13, Appendix I))

2.8.1 Gaps and Limitations

Several reports of alterations in immune function were located, however many of these were *in vitro* studies. Additional research on both humoral and cellular immunity, are required to establish dose-response relationships.

The lowest level of effect observed was by Setko (1989) (See Table A13, Appendix I). Exposures for one month to concentrations as low as 2 ppm H_2S containing natural gas (including hydrocarbon, mercaptan, and sulphur dioxide) appeared to produce significant alterations in humoral and non-specific immunity functions. The self-reports of increased immune symptoms (Legator et al., 2001) should be replicated in other low level H_2S -exposure settings. Concentrations of H_2S should be measured concomitantly with symptom surveys. Studies of mixtures such as natural gas, wastewater pond and landfill emissions should also be undertaken.

Prior research has shown that alkaline phosphatase is inhibited following H_2S inhalation in various tissues such as myocardium (Dwornicki, 1979), lung microsomes (Husain, 1976), kidneys (Kosminder et al. 1973), brain and liver when assayed in the absence of added magnesium (Hayden & Roth, 1988), and cerebellar cortex (Kosmider and Zajusz, 1966) (See Tables Appendix I). Placental alkaline phosphatase is involved in transport of functional antibodies to the fetus (Makiya and Stigbrand, 1992), however there were no recent studies that examined the effects of H_2S on this specific enzyme. Structural similarities among alkaline phosphatases in various tissues suggest that placental alkaline phosphatase may also be affected by H_2S exposure. Reduced transfer of immunoglobulin G to the fetus would reduce the immunological capacity of the newborn. Further studies are recommended to assess the effects of H_2S on transmission of antibodies to the fetus.

2.9 EFFECTS ON MUCOSAL TISSUES

Within the past decade, many reports were located describing the effects of H_2S and methyl mercaptan on various tissues such as dermal, periodontal, gastrointestinal, and ocular mucosa (Tables 13, 14, 15, 16, respectively). Effects of H_2S on respiratory mucosa are described in Section 2.14.

2.9.1 Dermal Effects

Few studies were located on the effects of H_2S on skin during the past decade or before. A high incidence of skin diseases was found in 75.9% of 758 workers of the Astrakhan gas processing plant occupationally exposed to natural gas processing products with a high content of hydrogen sulphide (up to 25%) (Rasskazov et al., 1996). One of six men, whose exposure was estimated at 8 - 16 ppm H_2S, had peeling facial skin (Tvedt et al., 1991a).

Arenberger (1999) and Arenberger and Schwarz (1995) investigated the effect of mineral waters containing H_2S on receptors for growth factor on human epidermal cells and for interleukin-8 on granulocytes. Using a radioligand, water-containing H_2S inhibited the binding of the epidermal growth factor and interleukin-8, due to a reduced affinity and number of receptors on the cell surface (Arenberger et al., 1995). In keratinocytes of patients with psoriasis, balneotherapy (therapeutic baths) produced a decline in the number and affinity of epidermal growth factor receptors, suggesting a mechanism for normalization of pathologically elevated proliferation in psoriatic plaques (Arenberger, 1999).

Table 12 Immune System Effects

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Sadowska et al. (1999) Immunological parameters were assessed in landfill workers in Belgium by multiparameter flow cytometry.	Compared to controls, landfill workers showed slight but significant increased in leukocytes, neutrophils, lymphocytes, CD8+ lymphocytes and complement C3.
Khan et al. (1998b) Isolated bovine neutrophils were exposed to sulphur dioxide, sulfite, sulfate, and H_2S for 4 or 24 hr at 25C. Neutrophil respiratory burst and myeloperoxidase activity was measured.	Concentration- and duration- dependent decreases in respiratory burst were observed for cells exposed to sulfite and sulfide. 4-hr incubations with less than 10 mM sulfite or sulfide produced small reductions (10 - 20%) in the rates of respiratory burst activity and superoxide production, whereas 25 - 50 mM sulfite or sulfide produced marked inhibitions (60-80%). 24-hr incubations with 0.5 - 1 mM sulfite or sulfide inhibited the respiratory burst and superoxide production 90 - 95%; the MPO activity was also reduced 30%.
Stair et al. (1996) The health of 460 beef cattle and their calves within 3 miles was assessed after a pipeline leak of volatile components of crude sour petroleum, emissions from burning sour condensate and steam washing of gravel occurred at the Red Deer River.	Suppression of the immune system, evident as lymphoid hypoplasia in spleen and lymph nodes of calves 6 weeks of age and older was observed. Lack of cellularity in the lymph node cortex and lack of lymphoid follicular structures was observed.

Bochanovskii et al. (1995) The immune status of CBA mice kept 4 months in the air-polluted area near the Astrakhan Gas Complex was studied.	T-cell immunity (a reduction of thymic and splenic mass, of mature T-cell number in the spleen) was more sensitive to the pollutants than B-cells and mononuclear phagocytes.
Granlund-Edstedt et al. (1993) Neutrophil killing of a capsulated and non-capsulated variant of a Group B streptococcal strain was evaluated in the presence or absence of H_2S and under aerobic and anaerobic conditions. SDS-PAGE of C3 and IgG coated on the bacteria was also undertaken.	Killing of bacteria was equally efficient under aerobic and anaerobic conditions however the presence of sulfide, killing of the capsulated variant was significantly inhibited. No cleavage of IgG was found with 2 mM sulfide. However the beta-chain of C3b, C3bi and the C-terminal part of the alpha-chain of C3b1 was released by sulfide.
Persson et al. (1993) Neutrophils were exposed to various concentrations of sodium sulfide and their chemotaxis and degranulation were studied after activation.	Neutrophils had similar chemotaxis capacity under aerobic and anaerobic conditions. The migration was inhibited a minor extent by 1 - 2 mM sulfide. The release of lactoferrin and myeloperoxidase was the same under aerobic and anaerobic conditions and was not significantly affected by sulfide.
Granlund-Edstedt et al. (1991) Serum was exposed to 2 mM sulfide under anaerobic conditions and the capacity of sulfide to split disulfide bonds of 10 serum proteins was evaluated by SDS-PAGE and immunodetection.	Sulfide had a low capacity to split the disulfide bonds of most proteins however was very effective towards the third component of complement. Sulfide released the C-terminal region of the alpha chain from C3bi.
Khan et al. (1991) Rats in a chamber exposed to 50, 200 or 400 ppm H_2S for 4 hours. The animals were killed after exposure and pulmonary macrophages recovered by lavage. The pulmonary alveolar macrophages viability and their respiratory rates were measured.	Complete abolition of zymosan-induced stimulation of respiratory rates of pulmonary alveolar macrophages after a 4 hour exposure to 200 or 400 ppm H_2S. No effects were seen at 50 ppm for either endpoint, however when the macrophages were stimulated with zymosan in vitro, a statistically significant reduction in the respiratory rate was seen for macrophages exposed to 30 uM sulfide and 30 uM sulfite. Also, in vitro, 30 uM sulfide inhibited basal respiratory rates compared to untreated control pulmonary alveolar macrophages.

Anecdotal reports suggest that skin is affected by sour gas exposures (Kostuch, 1999; Ludwig, 1997; Nikiforuk, 2001; p. 104). Several studies indicate that collagen synthesis is affected by mercaptans and H_2S. Lancero et al. (1996) and Johnson (1992 a,b; 1996) found that collagen synthesis was inhibited in human cultures of gingival fibroblasts exposed to 0.2 µM methyl mercaptan. The occurrence of 50% of one litter of H_2S -exposed rats with an epidermolysis bullosa-like condition (Dorman et al., 1999) may be due to lysyl oxidase inhibition.

Anecdotal reports also describe effects of H_2S-containing emissions on hair (Harris, 1992; McGlynn 1992; Kostuch 1992; Church, 1992). The hair was described as having an abnormal, faded or graying color, blotching, and rough and dry texture[15].

2.9.2 Periodontal Effects

A few studies have been undertaken in the past decade addressing the effects of reduced sulphur compounds on periodontal tissue (Table 14). Compared to control communities, a 6-fold increase in symptoms affecting the teeth and gums were reported by citizens living near wastewater treatment ponds or in a geothermal area (Legator, 2001). The 'teeth and gums symptoms' in the questionnaires included excessive dental cavities, excessive tooth loss, swollen or sore gums, toothaches, and other problems.

In a literature review, Ratcliff and Johnson (1999) summarized the effects of H_2S and methyl mercaptan in periodontal disease. These two gases are primarily responsible for mouth odour, although H_2S is the predominant volatile sulfur compound found (Persson, 1992). Peak concentrations were 1.9 mM H_2S and 0.16 mM methyl mercaptan that increased with the depth of the periodontal crevice (Persson, 1992; Colin and Tonzitich, 1992; Yaegaki and Sanada, 1992). Direct exposure to either reduces protein synthesis in cultured human gingival fibroblasts (Johnson and Lancero, 1999; Lancero et al, 1996; Johnson et al., 1992a, 1992b), however methyl mercaptan had the greatest effect. Crevice sulfide levels were increased with severity of periodontal disease, categorized by radiographic bone loss (Morita and Wang, 2001a). The highest concentration measured was ~1-3 ppm sulfide, which was in the range known to affect periodontium health (Morita and Wang, 2001a).

Other effects of methyl mercaptan included decreased intracellular pH (Johnson and Lancero, 1999; Lancer et al., 1996); inhibition of DNA synthesis (Johnson et al., 1992); less collagen synthesis (Johnson et al., 1996), more collagen degradation (Johnson et al, 1996), abnormal monomeric fibronectins (Johnson and Lancero, 1999); inhibition of proline uptake (Johnson et al., 1996; Johnson et al., 1992a); reductions in mature alpha 1 and alpha2 chains of Type I collagen and in Type III procollagen (Johnson et al., 1996; Lancero et al, 1996); accumulation of poorly-crosslinked collagen precursors which are susceptible to proteolysis (Johnson et al., 1992b), increased mucosal permeability, stimulation of cytokine (interleukin-1) production

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[15] Recall that hair, fur, hoof and nails have a relatively high proportion of disulfide-bonded proteins (keratins). Also recall that tyrosinase, the principal enzyme of melanin biosynthesis, has two coppers at its active site, making it susceptible to inhibition by H_2S.

(Ratkay et al., 1995); enhanced prostaglandin E2, cAMP and procollagenase secretion (Ratkay et al., 1995); and increased cell death (Johnson et al., 1992a).

Increased mucosal permeability was found upon exposure to nanomolar concentrations of methyl mercaptan, which was attributed to deaggregation by cleaving disulfide bonds in the extracellular matrix (Ng and Tonzetich, 1984). This rationale was supported by prior findings that blocking cysteine residues inhibited proteoglycan aggregation and that deaggregation of the matrix could be induced by disulfide-cleaving agents such as dithiothreitol. Indeed, many types of collagen, specifically III, IV, VI, VII, and IX are crosslinked by disulfide bonds (Goldberg and Rabinovitch, 1988). As type III collagen is prominent in basal laminae, the barrier layer of connective tissue at the basal surface of epithelial cells that limits exchanges of macromolecules between connective tissue and other tissues, disruption of its structure would allow leakage of various molecules.

2.9.3 Gastrointestinal Effects

In the past decade, a number of studies were located that examined the relationship between H_2S and gastrointestinal mucosa (Table 15).

Rat cecal mucosal homogenates were incubated with gas containing H_2S, methanethiol (CH_3SH), and dimethylsulfide, to determine if methylation occurs (Levitt et al., 1999). Neither CH_3SH nor dimethylsulfide was produced during H_2S catabolism, whereas catabolism of methanethiol liberated hydrogen sulphide but not dimethylsulfide. The authors concluded that H_2S and CH_3SH are not detoxified by methylation, rather that CH_3SH is demethylated to H_2S and H_2S is converted to nonvolatile metabolites, primarily thiosulfate. Suarez et al. (1998) measured the production rate of sulphur-containing gases in the rat colon. The major sulphur-containing gases were H_2S, CH_3SH, and dimethyl sulfide with cecal accumulation rates of 2.6, 0.096 and 0.046 uL/min, respectively. H_2S production was dependent on dietary components: a six-fold reduction after fasting, and a five-fold increase after carrageenan ingestion was found. The cecal mucosa rapidly metabolized the H_2S and methanethiol via a non-methylating reaction.

H_2S release from fecal samples from 25 patients with ulcerative colitis and 17 controls was measured at 24-hour intervals (Levine et al., 1998). Compared to controls, H_2S release was 3 - 4 times greater at every measurement point in the ulcerative colitis samples. Symptomatic ulcerative colitis patients' fecal samples produced greater amounts of H_2S compared to asymptomatic patients.

Several investigators have shown that H_2S and methyl mercaptan inhibit short chain fatty acid oxidation (Roediger et al., 1993; Pitcher and Cummings, 1996) in human colonocytes (Babidge et al., 1998) and unfractionated animal colonocytes (Moore et al., 1997a). Sulfides appear to irreversibly inactivate short-chain acyl-CoA dehydrogenase through CoA-persulfide formation (Shaw and Engel, 1987). In rats with 'in-flow' bowel colostomies that were treated with 2 mL sodium hydrosulfide (10, 20, 30 mM) twice daily via the stoma for 4 or 90 days, there was a highly significant reduction in $^{14}CO_2$ production from both *n*-butyrate and glucose in all groups compared to the control in both acute and chronic experiments (Moore et al, 1996; 1997b).

However, there was no difference in histological appearance between groups and no evidence of acute inflammation in any specimen. In contrast, prolonged 4-hr perfusion of the rat colon with 0.2 - 1.0 mM sulfide produced increased mucosal apoptosis, goblet cell depletion, and superficial ulceration compared to control animals (Aslam et al., 1992).

Mucosal biopsies from the sigmoid rectum of 10 patients without cancer, polyps or inflammatory bowel disease were incubated with either sodium chloride, sodium hydrogen sulfide (1 mM), a combination of both sodium hydrogen sulfide and butyrate (10 mM), or butyrate (Christl, et al., 1996). Sodium hydrogen sulfide induced hyperproliferation. Increased labeling (19%) of the entire crypt was observed, which was reversed when samples were co-incubated with sulfide and butyrate. Relative to controls, increased amounts of fecal H_2S were found in 13 men who had previously undergone surgery for sigmoid colon cancer and who later developed new epithelial neoplasia of the colon (Kanazawa et al., 1996).

2.9.4 Ophthalmic Effects

A number of studies in the past decade (Table 16) have reported alterations in corneal epithelium or eye irritation following H_2S exposure supporting earlier studies (Table A17 in Appendix I). The corneal epithelium appears to be quite sensitive to low levels of H_2S. The lowest level of effects reported were increased eye symptoms in the range of 3 - 5 ppb, as long term (monthly) averages, by Martilla et al. (1994, 1995).

Bates et al. (1997) reported significant increases in the incidence of diseases of sense organs; for example disorders of the eye and accessory parts (lacrimal glands), cataracts, disorders of the conjunctiva and the orbit. These effects were found in Rotorua residents where median H_2S concentrations were previously found to be near 14 ppb.

These findings support those of the Finland researchers (Martilla et al., 1995; Martilla et al., 1994; Parra et al., 1991; Jaakkola et al., 1990) demonstrating ocular effects of H_2S in the range of 2 - 70 ppb. Compared to a reference community, Marttila et al. (1994) reported that children exposed to 4 ug/m3 H_2S (3 ppb) (2-month mean value) had increased eye symptoms, which was concentration-dependent. Marttila et al. (1995) also reported increased risks for eye symptoms during periods when total reduced sulfur levels ranged between 11 and 14 ug/m3. From other studies in the same vicinity, H_2S typically comprises about 2/3 of the total reduced sulfur, equating to about 7 ug/m3 or 5 ppb H_2S.

Residents of 3 rural communities (one near an intensive hog operation, one near two intensive cattle operations and a third without livestock operations that use liquid waste management systems) were surveyed to assess health symptom and quality of life indicators (Wing and Wolf, 2000). Residents living near hog operations reported episodes of burning eyes (5.58 times), and blurred vision (1.25 times) more frequently than the reference community. Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community.

2.9.5 Other Cellular Effects

One report of altered electrolytes was located in the past decade (Table 17). Serum potassium was, on average, at the upper end of the normal range in 124 blood samples collected from cattle exposed to emissions from the Rankin County, Mississippi, blowout (Edwards, 1992) supporting earlier observations (Ranus et al., 1985; see Table A18, Appendix I).

Carbonic anhydrase (CA) inhibition by sulfide is implicated in H_2S -induced apnea (Almeida and Guidotti, 1999). CA is inhibited by mercaptans (Schwimmer, 1969), by sulfide (Lindskog & Thorslund, 1968) and by H_2S in rat brain homogenate (Roth et al., 1997; Nicholson et al., 1998)[16]. From crystallographic studies of carbonic anhydrase, Mangani and Hakansson (1992) found that the hydrosulfide anion, due to the close similarity between HS^- and OH^-, replaces the native zinc-bound water/hydroxide leaving the tetrahedral metal geometry unaltered. CA, due to its disulfide bonds (Baird et al., 1997), which stabilize the conformation of the N-terminal domain, and an active site loop (Stams et al., 1996), is susceptible to cleavage and inhibition by H_2S.

Often in cases of acute H_2S poisoning the arterial pH, blood gases and electrolyte balance are abnormal. For human exposures, the pH, pCO_2, pO_2 and serum bicarbonate have all been reported to be below normal to normal, and the anion gap is frequently increased (Gregorakos, 1995; Horowitz et al., 1997; van Aalst et al., 2000), consistent with earlier findings (Stine, 1976; Peters, 1981; Audeau et al., 1985; Hoidal et al., 1986; Deng and Chang, 1987; Table A9; Appendix I). In blood taken about 1 month after the blowout, the anion gap was slightly elevated in a group of 124 cattle located about 1.5 miles from a sour well blowout (actual H_2S concentrations not available) (Edwards, 1992). The increased anion gap is likely due to the extra sulfide and its oxidation products, thiosulfate and sulfate.

This combination of blood gas results resembles a metabolic acidosis, indicates a base or bicarbonate deficit and is similar to the findings in patients administered CA inhibitors (Sherwin and Bruegger, 1984). These findings are consistent with the reversible, non-competitive inhibition of CO_2 hydration activity of CA and the competitive inhibition of the dehydration reaction by monovalent anions (Wyeth and Prince, 1977). In erythrocytes at peripheral tissues, and in proximal renal tubule cells, CA I and II catalyze CO_2 hydration to form bicarbonate at a rate of $8X10^5$ - 10^6/sec (Baird et al., 1997). The bicarbonate then is transported out of the red cell concurrent with chloride transport into the cell (chloride shift).

At the lungs, the reverse reactions occur. Bicarbonate is transported into the erythrocyte and its dehydration is catalyzed by CA IV, which is activated by low concentrations of chloride (Baird et al., 1997) corresponding to the influx of chloride during the chloride shift, to facilitate rapid CO_2 removal at the lung. The low pCO_2 in the alveoli allows carbon dioxide to diffuse out of the erythrocyte, across the capillary endothelial surface to pass out of the lung upon expiration. Inhibition of CA in erythrocytes and renal tubule cells slows the formation of bicarbonate that is used by the erythrocyte in the hemoglobin buffer system as well as the renal tubule cells (Sherwin and Bruegger, 1984, p 400), which eventually leads to an acidotic state. A decreased pCO_2 is not normally observed in an early metabolic acidosis, but as the body attempts to adjust, the pCO_2 decreases. Sulfide-induced CA inhibition in the erythrocyte, which catalyzes CO_2 hydration to form bicarbonate, and inhibition of CA in the lung, which normally catalyzes dehydration of serum bicarbonate to produce carbon dioxide, explains the decreased pCO_2 in H_2S -exposed individuals. Patients overdosed with calcium polysulfide that were treated with sodium bicarbonate intravenously or by lavage appeared to have better outcomes than those given sodium nitrite intravenously (Horowitz et al., 1997)[17].

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[16] Sulfide inhibition of CA in bovine erythrocytes is reported with a Ki 1.9 uM (Wyeth and Prince, 1977). Sodium sulfide (29 uM) inhibited carbonic anhydrase 84, 81 and 77% in the hippocampus, frontal cortex and cerebellum, respectively (Nicholson et al., 1998). Beef red blood cell carbonic anhydrase is inhibited 50% at 1.5 uM sulfide in vitro (Almeida and Guidotti, 1999).

2.9.6 Gaps and Limitations

Many of the studies discussed in this section address the effects of H_2S on the cellular level, which for some tissues (gastrointestinal tract, skin), due to their regenerative capacity, would be reversible. However, for tissues where the regenerative capacity is limited, the effects may be chronic or irreversible.

The lowest H_2S effect levels for mucosal tissues (eye) were reported by Martilla et al. (1994, 1995). Monthly average concentrations of H_2S of 3 - 5 ppb were associated with dose-dependent increases in eye symptoms in children and adults. There was some evidence that peaks of exposure may play a role (Martilla et al., 1990). Although the studies undertaken in other locations (e.g. Rotorua, hog operations) did not measure symptom prevalences simultaneously with air H_2S concentrations, they do suggest that eye irritation occurs at levels below 10 ppb.

Additional studies should be undertaken to assess eye symptom prevalence, visual acuity, and ocular diseases in areas of reduced sulfur sources such as pulp and paper mills and large-scale farm operations with simultaneous measurement of pollutant concentrations.

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[17] However, one of the poisoned patients who did not survive was also infused rapidly with normal saline by paramedics, which may further contribute to the metabolic acidosis because the high sodium load competes with hydrogen ions for renal excretion (Sherwin and Bruegger, 1984; p. 399).

Table 13 Dermal Effects: Human and Animal Studies

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95% CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Arenberger (1999) Keratinocytes obtained from patients with psoriasis exposed to sulphur baths were compared to cells obtained prior to treatment.	Epidermal growth factor receptors were reduced in number and binding affinity.
Cited in Searcy and Lee (1998)	Ointments containing elemental sulphur were applied to the skin and sometimes caused symptoms of H_2S poisoning.
Rasskazov et al. (1996)	A high incidence of skin diseases was found in 75.9% of 758 workers of the Astrakhan gas processing plant occupationally exposed to natural gas processing products with a high content of hydrogen sulphide (up to 25%).
Arenberger & Schwarz (1995) The effect of mineral water containing H_2S on epidermal growth factor receptor on human epidermal cells and for interleukin-8 on granulocytes was evaluated using radioligand-binding assays.	Water containing H_2S inhibited the binding of the epidermal growth factor as well as interleukin-8. Suppression of the binding of the cytokines was due to a reduced affinity and number of receptors on the cell surface.
Tvedt et al. (1991a) Six men lost consciousness after acute H_2S exposure.	One of six men whose exposure was estimated at 8 - 16 ppm had peeling facial skin.

Table 14 Periodontal Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Morita and Wang (2001a) The relationships between sulcular sulfide and severity of periodontal disease and between sulcular sulfide and the BANA test were examined in 70 patients.	The mean sulcular sulfide levels were 0.1 +/- 0.23, 0.36 +/- 0.48 and 1.10 +/- 0.78 for healthy, low-moderate, and severe periodontal disease (bone loss) sites, respectively. The sulcular sulfide levels correlated positively with the BANA test, which assesses the presence of *T. denticola*, *P. gingivalis*, and *B. forsythus*. The highest sulcular sulfide score was 2.5, which corresponded to a concentration of 1-3 ppm sulfide.
Morita and Wang (2001b) The relationship between malodor and sulfide levels in periodontal pockets was studied in 81 periodontal patients.	The volume of tongue coating, extent of periodontal disease, sulcular sulfide levels in sites with low to moderate bone loss, and percentage of sites with bleed on probing were significantly associated with oral malodor.
Ratcliff and Johnson (1999) Literature review	H_2S and CH_3SH are primarily responsible for mouth odour. Direct exposure to either reduces protein synthesis in cultured human gingival fibroblasts, however CH_3SH has the greatest effect. Other effects include less collagen synthesis, more collagen degradation, accumulation of poorly-crosslinked collagen precursors which are susceptible to proteolysis, increase mucosal permeability, and stimulate cytokine production.
Johnson and Lancero (1999) Human gingival fibroblasts and periodontal ligament cells were exposed to a continuous flow of CH_3SH *in vitro* to determine the effect of exposure on cell function.	A consistent decrease in intracellular pH was observed following exposure. Exposed periodontal ligament cell cultures produced about 30% less protein. Abnormal monomeric fibronectins were found in exposed cells.
Johnson et al. (1996) Human gingival fibroblast cultures were exposed for 30-min to 10 ng CH_3SH /ml in air mixture above the culture medium.	A short exposure of CH_3SH suppressed collagen synthesis by 39%, increased the intracellular degradation of newly synthesized collagen from 26 - 42%, and inhibited proline uptake by 29%. After a 12-hr exposure, a marked increase in extracellular collagenolysis (4% in control vs. 55% in test) and an increase in intracellular degradation (20% control vs. 30% test) were reported. Reductions in both mature alpha 1 and alpha 2 chains of Type I collagen and in Type III procollagen was observed.

Lancero et al. (1996) Human periodontal ligament cells obtained fresh from 3 healthy adults were grown in tissue culture (with or without 10 ng/mL CH_3SH in air mixture) for 48 hrs. Intracellular pH was measured before, during and after acid loading. Total protein, electrophoresis and cell migration and cell viability assays were also done.	Resting intracellular pH levels were significantly lower in CH_3SH exposed cells (6.84, 6.85) compared to controls (7.31, 7.20). Compared to controls (2.4 % non-viable), a small increase in non-viable cells was found in CH_3SH exposed cells (8.5%). Methylmercaptan also significantly inhibited protein synthesis (~30%), inhibited cell migration, and reduced the amounts of mature 1 and 2 type I collagen chains.
Ratkay et al. (1995) Human gingival fibroblasts were exposed to CH_3SH alone or in combination with interleukin-1 (IL-1) or lipopolysaccharide (LPS).	Significantly enhanced secretion of prostaglandins E2, cAMP and procollagenase was found. Mononuclear cells were stimulated by CH_3SH to produce IL-1, which can increase cAMP production and act in synergy. CH_3SH also significantly enhanced the activity of cathepsin B, moderately suppressed cathepsin G, but did not affect elastase.
Persson (1992) Gingival fluid was collected on paper strips from patients and staff of the School of Dentistry and subsequently analyzed by GC-FID.	H_2S is the predominant volatile sulphur compound found in periodontal pockets, found in 61 out of 79 patients studied. Peak concentrations were 1.9 H_2S and 0.16 mmol CH_3SH /liter and the amount of H_2S and CH_3SH increased with the depth of the periodontal crevice.
Coli and Tonzetich (1992); Yaegaki and Sanada (1992); Ratcliff and Johnson (1999) Literature reviews	The presence of volatile sulphur compounds (H_2S, CH_3SH, dimethylsulfide and dimethyl disulfide) in gingival crevices was associated with increased gingival crevice depth, with gingival inflammation and with periodontal disease.
Johnson et al. (1992a) Human gingival fibroblast cultures and viable porcine unkeratinized oral mucosal tissue sections were exposed to CH_3SH.	DNA synthesis was inhibited by 44%, protein content decreased by 25%, which was not reversible upon removal of CH_3SH. Alterations in collagen metabolism were found (pooling of Type I collagen). A 15 -min. exposure at 6.7 ng/ml, reduced proline transport by 24%, and increased cell death was observed in exposed tissue sections.
Johnson et al. (1992b) The effects of H_2S and CH_3SH on protein metabolism of human gingival fibroblasts were evaluated by measuring the rate of labeled amino acid incorporation. Collagenous protein concentrations were also measured.	Both gases reduced total protein synthesis with CH_3SH exerting a larger effect. The changes in total protein following methyl mercaptan and H_2S exposure were accompanied by a corresponding decrease in collagenase-digestible protein. The methyl mercaptan exposed cultures had a 70% reduction in collagen, which resulted from a combined effect of suppressed synthesis and increased rate of collagen degradation.

Table 15 Effects on Gastrointestinal Mucosa

Reference / Study Design	Key Findings
Levitt et al. (1999) Rat cecal mucosal homogenates were incubated with gas containing H_2S, CH_3SH, and dimethylsulfide, to determine if methylation occurs.	Neither CH_3SH nor dimethylsulfide was produced during H_2S catabolism, whereas catabolism of methanethiol liberated H_2S but not dimethylsulfide. The authors concluded that H_2S and CH_3SH are not detoxified by methylation, rather that CH_3SH is demethylated to H_2S and H_2S is converted to nonvolatile metabolites, primarily thiosulfate.
Levine et al. (1998) H_2S release from fecal samples from 25 patients with ulcerative colitis and 17 controls was measured at 24-hr intervals.	Compared to controls, H_2S release was 3 - 4 times greater at every measurement point in the ulcerative colitis samples. Symptomatic ulcerative colitis fecal samples produced greater amounts of H_2S compared to asymptomatic patients.
Suarez et al. (1998) The production rate of sulphur-containing gases in the rat colon was measured.	The major sulphur-containing gases were H_2S, CH_3SH, and dimethyl sulfide with cecal accumulation rates of 2.6, 0.096 and 0.046 uL/min, respectively. H_2S production was dependent on dietary components - a 6-fold reduction after fasting, and a 5-fold increase after carrageenan ingestion was found. The cecal mucosa rapidly metabolized the H_2S and CH_3SH via a non-methylating reaction. Based on the measured turnover rate, the mucosal exposure was determined to be 10 times greater than the measured accumulation rate.
Babidge et al. (1998) Isolated human colonocytes from cases without colitis were exposed to 1.5 mM sulfide in the presence or absence of exogenous CoA and ATP. Short chain acyl-CoA esters were measured by HPLC.	1.5 mM sulfide inhibits short chain acyl-CoA dehydrogenase in colonocytes isolated from patients without colitis. A block in beta-oxidation of short chain fatty acids in colonic epithelial cells is associated with the disease process of ulcerative colitis.
Moore et al. (1997a) Rat colonic epithelial cells were incubated with C14-n-butyrate (5mM) with and without 1.5 mM sodium hydrosulfide (NaHS), and both in the presence and absence of exogenous CoA and ATP. Metabolic function was assessed by $^{14}CO_2$ production and by acyl-CoA ester production.	Colonocytes incubated in the presence of exogenous CoA and ATP, treatment with NaHS significantly diminished $^{14}CO_2$ production. This was associated with an increase in butyryl-CoA and a reduction in crotonyl-CoA concentrations. There were no significant differences in acyl-CoA ester profiles observed when cells were incubated in the absence of exogenous CoA and ATP.

Moore et al. (1997b) Adult Sprague-Dawley rats with 'in-flow' bowel colostomies were treated with 2 mL sodium hydrosulfide (10, 20, 30 mmol/L) twice daily via the stoma for 4 (acute) and 90 (chronic) days. Isolated colonic epithelial cells prepared from the animals were incubated in the presence of ^{14}C-labelled n-butyrate (5 mmol/L) or ^{14}C-glucose (5 mmol/L). Metabolism was assessed by $^{14}CO_2$ production and enzymatically by ketone body and lactogenesis production. The mucosa histological appearance was scored for acute inflammatory changes.	There was a highly significant reduction in $^{4}CO_2$ production from both n-butyrate and glucose in all groups compared to the control in both acute and chronic experiments. There was no difference in histological appearance between groups and no evidence of acute inflammation in any specimen.
Moore et al. (1996)	Experiments with spiked installation of sulfide into the proximal rat colon produced diminished n-butyrate oxidation but no histological changes.
Kanazawa et al. (1996) 13 men who had previously undergone surgery for sigmoid colon cancer and who later developed new epithelial neoplasia of the colon were compared with controls whose large bowel was entirely normal by total colonoscopy.	Fecal pH and amounts of H_2S and cresol were higher in the cases than the controls.
Christl et al. (1996) Mucosal biopsies from the sigmoid rectum of 10 patients without cancer, with polyps or inflammatory bowel disease, were incubated with sodium hydrogen sulphide (1 mM) or with a combination of sodium hydrogen sulphide and butyrate (10 mM) to study the effects of sulfide on mucosal cell proliferation.	Sulfide increased the proliferation zone by 54% in the upper crypt, which was inhibited by butyrate.

Roediger et al. (1993); Pitcher and Cummings (1996) The metabolic effects of various mercaptides (sodium hydrogen sulphide and sodium methanethiol) were evaluated in human colonocytes isolated from 31 colectomy specimens.	Both mercaptides, sodium hydrogen sulphide greater than sodium methanethiol, were found to be injurious at all sites in the colon. Significant inhibition of n-butyrate but not glucose oxidation by sodium hydrogen sulphide was found in the ascending colon, splenic flexure and rectosigmoid regions. Fatty acid oxidation was inhibited more by hydrogen sulphide in the rectosigmoid region than in the ascending colon. Fatty acid oxidation in colonocytes is inhibited, in order of suppression, by H_2S, CH3SH, and mercaptoacetate.
Roediger et al. (1993) Literature review	Sulphur-containing gases have been pathogenetically implicated in ulcerative colitis.
Aslam et al. (1992)	Prolonged 4-hr perfusion of the rat colon with 0.2 - 1.0 mM sulfide produced increased mucosal apoptosis, goblet cell depletion, and superficial ulceration compared to control animals.

Table 16 Ophthalmic Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Wing and Wolf (2000). Residents of 3 rural communities (one near an intensive hog operation, one near two intensive cattle operations and a third without livestock operations that use liquid waste management systems) were surveyed to assess health symptom and quality of life indicators.	Residents living near hog operations reported episodes of burning eyes (5.58 times), and blurred vision (1.25 times) more than the reference community. Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community.
TNRCC (1998) Six workers were exposed to a mean concentration of 0.09 ppm H_2S for approximately 5 hrs in a monitoring van downwind from an oil refinery	Persistent odors, eye and throat irritation, headache and nausea were observed in the workers.
Bates et al. (1998) A retrospective study using hospital discharge data from 1981 - 1990 was compared to rates of the rest of New Zealand. Rotorua sits on a geothermal field that has continuous ambient low-level H_2S (median conc. 20 ug/m3; 14 ppb) and mercury.	Statistically significant increases in the incidence of diseases of the nervous system and sense organs (SIR 1.11); for other disorders of the eye and adnexa (SIR 1.12); for cataract (SIR 1.26); disorders of the conjunctiva (SIR 2.09) and disorders of the orbit (SIR 1.69) were found.
Marttila et al. (1995) This was a longitudinal study of respiratory symptoms in a community living near a pulp mill. Pollutant levels were obtained by continuous monitors or dispersion models.	Significantly increased risks for eye [OR 3.17; 95%CI 1.21 - 7.47], nose and throat symptoms were reported during episodes of when TRS levels ranged between 11 - 14 ug/m3. [In other studies in the same vicinity, H_2S typically comprises about 2/3 of TRS].
Anonymous (1995) Investigators of the National Institute for Occupational Safety and Health conducted a health hazard evaluation at a wastewater treatment plant in Independence, Missouri.	Employees reported eye irritation and other complaints while working in the belt pressroom. Measurements of personal breathing zone concentrations of H_2S were: maximum 10-minute ranged from 0.1 ppm to 95 ppm; 8 of the 13 personal breathing zone samples exceeded 10 ppm and 3 exceeded 20 ppm. Maximum 10-min concentrations in general air samples obtained in the belt pressroom range from 46 - 69 ppm whereas outside the belt pressroom, the maximum 10-min concentrations ranged from non-detectable to 0.1 ppm.

Marttila et al. (1994) A cross-sectional long-term study of children's health and pulp mill emissions. Parents of 134 children from three communities were surveyed.	Compared to the reference community, increased risks of nasal symptoms (OR 1.4), cough (OR 1.83), eye symptoms (OR 1.15) and headache (1.77) were found in the severely polluted community. The measured 2-month mean H_2S concentration in the reference, moderately-, and severely polluted community was not detectable, 2, and 4 ug/m3, respectively.
Parra et al. (1991) Case report of workers exposed to H_2S from a toilet facility. Two days after the incident, H_2S was measured and was not above the threshold limit value (10 ppm).	One man noticed eye, nose, and throat irritation when he entered the toilet facility, which disappeared after an hour. On the same day, 3 other workers were admitted to hospital because of nausea, vomiting dizziness and dyspnea; one died a few hours later. Ten more workers reported nausea, vomiting, itchy eyes, and nose irritation and recovered without any further problems.
Lefebvre et al. (1991) A method for measuring eye irritation is described.	The exfoliative eye cytology procedure was shown to give quantitative information on the degree of ocular irritation in rats.
Jaakkola et al. (1990) A survey of people living near a paper mill (mean annual hydrogen sulphide exposure was estimated at 4.3 ppb; methyl mercaptan mean annual concentration 2 - 5 ug/m3; 1 - 2.5 ppb)	People living near the mill reported 12 times more eye irritation than people without exposure. Irritation effects may have been related to peak concentrations, which were as high as 70 ppb for H_2S and 50 ug/m3 (25 ppb) for methyl mercaptan.
Vanhoorne et al. (1990) [cited in Gangolli, 1999]	Viscose rayon washers exposed to up to 9 mg/m3 (6.4 ppm) showed associated complaints of eye irritation at levels below 14 mg/m3 (10 ppm).

Table 17 Other Cellular Effects

Reference / Study Design	Key Findings
(Edwards, 1992) Electrolytes were measured in blood collected from cattle exposed to emissions from the blowout in Rankin County, Mississippi.	Serum potassium was, on average, at the upper end of the normal range in 124 blood samples collected

2.10 NERVOUS SYSTEM EFFECTS

Many studies have been undertaken in the past decade, which have helped elucidate some of the effects of H2S on the nervous system (Tables 18 and 19). In studies of human exposure, a variety of effects on the nervous system have been reported. Alterations in neurophysiological and neuropsychological tests and increased frequencies of symptoms have been found following both short-term high-level exposures and long-term low-level exposures.

2.10.1 Human Studies

Schneider et al. (1998) described a case following long-term exposure to H_2S exposure in a previously healthy 27-year old male construction worker. The man was a popular, well-adjusted, conscientious student who performed above grade level on academic indices. He was part of a crew that was building a sewer system in a New Jersey wetland and was exposed to H_2S as he descended a ladder into the 27-foot-deep pit to rescue a co-worker. Measurements at the site four hours after the event indicated concentrations of 22 ppm H_2S at the surface of the water (Anonymous, 1993). The vibrational construction activity underway at the site at the time of the injury would have significantly increased the H_2S concentration.[18] He fell an unknown distance but there was no evidence of head trauma (skin abrasions, bruising, normal head CT scan), yet he experienced multiple and severe neurological deficits affecting the left thalamus, basal ganglia, temporal and inferior parietal lobes, putamen and the amygdala/hippocampal region (See Table 18 for details).

Similar regions of the brain (basal ganglia, putamen, caudate nucleus, occipital lobe cerebellum, and cortex) were affected in Rhesus monkeys (Lynd and Wielund, 1966; SeeTable A19; Appendix I) and in H_2S exposed workers who developed clinical encephalopathy (Callender et al., 1993). Seven of the 9 workers exposed to H_2S (and in all cases, other substances) had abnormal single-photon emission computed tomography (SPECT) neuroimaging results. The scans of the cerebellum were abnormal for 2/9 workers; the frontal lobe 4/9, temporal lobe 3/9; thalamus, 1/9; cerebrum 1/9 and basal ganglia 5/9 workers. Neuropsychological testing was abnormal in 4/6 workers, with deficits occurring the attention and mental flexibility (3/6), learning and memory (2/6), motor (2/6) and visual-spatial (1/6) domains. Neurophysiological testing showed abnormalities of color vision in 6/7 workers; olfaction in 5/8 and current perception threshold in 7/9.

Of interest, is the consistency noted between the brain areas affected in these 3 studies and structural locations where high cytochrome c oxidase activity has been mapped in rats. High activity is present in the basal ganglia (caudate-putamen and nucleus accumbens), the cerebellar cortex, parietal cortex, hypothalamus mammary bodies, specialized sensory areas (olfactory bulb, visual, and auditory domains), and motor areas the frontal cortex (Hevner et al., 1995).

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[18] In another case report of fatality in a swamp area, three men attempting to clean a well in a swamp area died from short exposures to H_2S in the well atmosphere. After duplication of the conditions was attempted, concentrations of 210, 220, and 250 ppm H_2S were found at levels of 1 ft, 8 ft, and 18 ft. below the ground level. (McDonald, 1951).

As a part of a health survey, 187 viscose rayon workers in Belgium exposed to CS_2 and H_2S for at least one year underwent an extensive neuropsychological examination (DeFruyt et al., 1998). Sixty-seven workers served as non-exposed controls. The CS_2 concentration ranged from 3-147 mg/m^3 (1-47 ppm) and H_2S was below 14 mg/m^3 (10 ppm). The neuropsychological investigation included subtests of the Weschler Adult Intelligence Scale, the entire Wechsler Memory Scale, the Bourdon-Wiersma Test, the Santa Ana Dexterity Test, the Gibson Spiral Maze, and the Bimanual Sinusoidal Movement Test. Only the group exposed to values exceeding three times the recommended limit for CS_2 (31 mg/m^3; 10 ppm) exhibited significant impairments in both the speed and quality of psychomotor performance. Exposure to CS_2 and H_2S had no significant effect on memory and attention.

In contrast, Kilburn (1999) found abnormal neurophysiological and neuropsychological test parameters among residents living near a desulphurization unit and among workers previously employed at the site. In the lowest exposure group (H_2S concentrations ranged from 0.1 - 1 ppm with occasional peaks of 5 ppm), abnormalities were found in balance, colour discrimination, grip strength and verbal recall. Kilburn reported similar findings in 1995, 1997 and 1998. Compared to controls, test results for balance, choice reaction time, and immediate story recall were found to be abnormal for various exposed groups. The frequency of symptoms affecting memory, balance, mood, and limbic functions were also increased relative to the control group. Workers formerly employed in a sour crude oil refinery and residents living within 1.2 km of the plant exhibited impaired reaction time, balance, colour discrimination, trail making, and immediate story recall (Kilburn and Warsaw, 1995). Anger, confusion, depression, tension-anxiety and fatigue scores were also significantly elevated compared to controls. Personal exposures were not measured, however 24 hr averages of gases monitored outside the desulphurization unit were 0 - 8.8 ppm H_2S, and 6.1 - 70.1 ug/m^3 total reduced sulphur. The average H_2S concentration was 10 ppb with peaks of 100 ppb.

Similarly altered mood states were reported by Schiffman et al. (1995) and Haahtela et al. (1992). In the Schiffman study, the effect of environmental odors emanating from a large hog operation on the mood of 44 residents living nearby was determined using the Profile of Mood States (POMS). They were compared to 44 control subjects who were matched according to gender, ethnic group, age and years of education. A significant difference between control and experimental subjects for all six POMS factors and the total mood disturbance score was found. People living closest to the intensive swine operation who experienced the odors had significantly more tension, depression, anger, fatigue, confusion and less vigor than controls. People exposed to the odors also had greater total mood disturbance than controls as determined by their ratings on the POMS. In the Haahtela study, significantly increased symptoms of increased mental symptoms (depression and anxiety) were found in 10% of 75 subjects living downwind of a paper mill that released H_2S and mesityloxide for two days. The 24-hr average H_2S concentration for the two days was 35 and 43 ug/m^3 (25 ppb, 30 ppb); a 4-hr maximum was 135 ug/m^3 (96 ppb); mesityloxide was not measured.

Postural stability has repeatedly reported to be altered by H_2S exposure (Kilburn et al., 1995, 1997, 1998, 1999) supporting earlier reports (Hirasawa, 1976; See Table A7.10.1; Appendix I). Postural stability was measured in 28 sewer workers who have worked at the plant at last 6 months (Kuo et al., 1996). Volatile organic solvent concentrations, measured with a photoionization detector as benzene equivalents were also measured. The mean (+/- SD) solvent concentration was 0.32 +/- 0.19 ppm benzene equivalent, range 0.02 - 0.95ppm. Compared with a non-exposed population, the workers had increased postural sway. Statistically significant positive correlations were found between postural sway (r = 0.91 for sway length; 0.72 for sway area) and organic solvent exposure. Yet the solvent exposure was very low and the authors suspected that some other agent, rather than the solvents might be responsible. It is surprising, given the prior findings of altered postural sway among H_2S -exposed individuals by Kilburn et al. (1995) and Hirasawa (1976), that these investigators did not consider the possible effects of reduced sulfur compounds in their analysis or discussion.

Reports of neurological symptoms such as headache, dizziness, and fatigue are common around H_2S sources (Legator et al., 2001; Wing & Wolf, 2000; Thorn and Kerekes, 2001; Vrijheid, 2000; TNRCC, 1998; Berger, 1996 [cited in McGavran, 2001]; Partti-Pellinen et al., 1996; Pach et al., 1996; Kilburn and Warsaw, 1995; Anonymous, 1995; Marttila et al., 1994b; Haahtela et al., 1992; Jappinen et al., 1990). Anecdotal reports of these symptoms are also common (Nikiforuk, 2001).

A cross-sectional study of 336 adults living near a pulp mill and of 380 adults in a reference community, was undertaken by Partti-Pellinen and colleagues (1996). After controlling for confounders, compared to the reference community, increased risks for headache or migraine in the previous 4 weeks (OR 1.83; 95% CI 1.06 - 31.5) or past 12 months (OR 1.70; 95% CI 1.05 - 2.73) were found. In the exposed community, the annual mean TRS and SO_2 concentrations measured were 2-3 ug/m3 and 1 ug/m^3, respectively. In the reference community, the SO_2 concentration was 1 ug/m^3.

Legator et al. (2001) found significantly increased neurological symptoms (OR 12.7; 95%CI 7.59 - 22.09) among populations exposed to emissions from a geothermal plant (Puna) and an industrial wastewater pond (Odessa). In the Puna group, fatigue was reported the most frequently (~61%), followed by difficulty sleeping and anxiety, change in senses (both ~53%), short term memory loss and depression (both ~50%), lethargy and headaches (~47%), numbness (~42%), and restlessness, balance, dizziness (~34%). Increased symptoms were also reported in the Odessa group, albeit not as frequently. The authors noted that several neuropsychological tests that were performed on exposed subjects supported the findings within the nervous system symptom category. Prior air monitoring during 1996 - 1997 showed levels in the 'low ppb range', although most hourly measurements were less than 1 ppb or were not detectable. During that same time period, 29 incident reports showed a peak concentration of 301.7 ppb. In other years, concentrations of 200 - 500 ppb have been recorded.

Residents living near hog operations reported episodes of headache 7.6 times and blurred vision 1.25 times more than the reference community (Wing and Wolf, 2000). Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community. Residents living near cattle operations reported episodes of headaches 1.57 times more than the reference community.

Residents living near oil batteries in the Tilston, Manitoba area reported nausea 4.2 times more frequently and headaches 1.5 times more frequently than the control group (Kraut, 2000). Air sampling at the battery showed ten 1-hr readings above the provincial guideline of 11 ppb with a peak of 36.0 ppb and on one of the farms, lower levels were detected. Volatile organic compounds were also measured at the battery and found to be 'usually lower' than those measured in Winnipeg. On Dec 12, 1993, during a well-testing operation near the Ludwig farm northeast of Grande Prairie, Alberta, 1694 m^3 raw gas containing 3.2% H_2S was released into the air without flaring it (Nikiforuk, 2001; p. 38). The estimated H_2S concentration, made by Ranchmen Resources Ltd. staff, was that the Ludwig family were exposed to between 2 and 4 ppm H_2S. Shortly after, the Ludwig's were nauseated, vomiting and had throbbing headaches.

Air testing was done near 17 large-scale hog manure lagoons, some as large as a city block, in Renville County by Land Stewardship Project members and staff after area residents complained of nausea, headaches, blackout periods, vomiting and other symptoms (Anonymous, 1996). During a 2-week period in May, a Jerome Analyzer was used to measure H_2S concentrations near the lagoons as well as up to 1.5 miles away. All of the sites showed some H_2S in the air. Two of the tests showed mean H_2S levels of more than 100 ppb, with one site measuring 134 ppb. Eight of 32 tests showed mean H_2S concentrations of more than 50 ppb.

In Rotorua, a geothermal area in New Zealand, increased standardized incidence ratios (SIR) for diseases of the nervous system and sense organs (SIR 1.11; 95%CI 1.07 - 1.15) were found (Bates et al., 1998). Further classification gave increased ratios for other disorders of the central nervous system (SIR 1.22; 95%CI 1.11 - 1.33); disorders of the peripheral nervous system (SIR 1.35; 95% CI 1.21 - 1.51), infantile cerebral palsy (SIR 1.42; 95%CI 1.03 - 1.89); migraine, (SIR 1.40; 95% CI 1.12 - 1.72); other brain conditions (SIR 2.50; 1.89 - 3.26); mononeuritis of upper limb and mononeuritis multiplex (SIR 1.47; 1.29 - 1.67); and mononeuritis of lower limb (SIR 2.06; 1.46 - 2.81). Although the median H_2S concentration previously measured was 14 ppb, other geothermal gases such as mercury or radon may be present (no measurements were given).

In a literature review, Inoue (1993) identified H_2S as a factor in a movement disorder similar to Parkinsonism. Defects in mitochondrial respiration (Complex I - NADH dehydrogenase activity) and the monoamine oxidase (MAO) pathway are believed to contribute to the development or progression of this disease. The proposed explanation is that MAO-generated hydrogen peroxide oxidizes glutathione to glutathione disulfide (GSSG), which undergoes thiol-disulfide interchange to form mixed disulfides, thereby interfering reversibly with thiol-dependent enzymatic function (Cohen et al., 1997). Evidence in support of this includes the direct addition of GSSG to mitochondria resulted in similar reversible inhibition of electron transport and an elevation in mixed protein disulfides within mitochondria induced by monoamines (see references in Cohen et al., 1997).

The physiological and biochemical states of children and adults who resided in the vicinity of the sulphide-containing gas processing plant were studied (Boev et al., 1998). The chemical agents emitted by the plant were found to have adverse effects of the children's functional status, namely, decreased vital capacity of the lung, mental performance, retarded sensory-motor responses, altered enzymatic system activity.

2.10.2 Animal Studies

2.10.2.1 Lipid Alterations

In the past decade, only one study was located that looked at the effects of H_2S on lipid metabolism. Boev et al. (1992) reported increased lipid peroxidation in cerebral cortex of rats exposed to sour gas containing H_2S. This finding supports earlier work by Haider and Hasan (1984) and Haider et al. (1980) (See Table A20; Appendix I).

2.10.2.2 Neuromodulation

Abe and Kimura (1996) have shown that physiological concentrations of sodium hydrosulfide (10 - 130 μM) selectively enhance N-methyl D-aspartate (NMDA) receptor-mediated responses and facilitate the induction of hippocampal long-term potentiation (LTP). These findings suggest that endogenous H_2S functions as a neuromodulator. They demonstrated that cystathionine beta-synthase (CBS)[19] in rat brain produces H_2S. The expression is higher in the hippocampus and cerebellum than in the cerebral cortex or brainstem. In homogenized whole brain, H_2S was produced at a rate of 22.6 +/- 1.6 nmol/min per gram of protein. This reaction was inhibited by known CBS inhibitors and was enhanced by known activators (S-adenosylmethionine). The effect of H_2S on synaptic transmission, on long-term potentiation and its interaction with NMDA receptors was further characterized. NaHS concentrations less than 130 μM did not affect the synaptic transmission in the hippocampus but higher concentrations (320 and 640 μM) were inhibitory. They also found that the physiological concentrations of H_2S facilitated the induction of LTP only when it was simultaneously applied with a weak tetanic stimulation, which was completely occluded by strong tetanic stimulation. When considered together, these results indicate that the H_2S-induced LTP shares a common mechanism with LTP induced by a strong tetanic stimulation. To determine whether the facilitation of LTP by H_2S required NMDA receptor activation, the effect of NaHS on LTP induction in the presence of an NMDA receptor agonist was studied. The results suggested that the induction of LTP by H_2S requires the activation of NMDA receptors, confirmed by whole-cell patch recordings of NMDA-induced currents. The enhancing effect of H_2S on NMDA response was found to be selective for the NMDA receptors, to be concentration-dependent in the range of 10 - 130 μM, and was not related to the status of NMDA receptor thiol redox groups.

These findings were supported by *in vitro* studies of primary cultures of rat brain cells (neural and glial) induced by sodium hydrosulfide to produce 3',5' cyclic adenosine monophosphate (cAMP) in the presence and absence of inhibitors (Kimura, 2000). Physiological concentrations of sodium hydrosulfide induced cAMP production in primary cultures of central nervous system

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[19] Cystathionine B synthase (CBS) activity, which is believed to be the enzyme producing H_2S in the brain (Abe & Kimura, 2000) is highly regulated (Mosharov et al., 2000), tissue-specific (Quere et al., 1999), present in brain and adipose tissue, absent in heart lung, testes, adrenal and spleen (Finkelstein, 1990) and compartmentalized in the endoplasmic reticulum (Allsop & Watts, 1975). These characteristics strongly suggest that any alterations in production, dissemination or consumption of the enzyme, products or substrates could have potentially damaging outcomes. This is the case for people with deficiencies of CBS. CBS deficiency is an inherited metabolic disease characterized by dislocated eye lenses, skeletal problems, vascular disease and mental retardation (Mudd et al., 2001).

[CNS] neurons and neuronal and glial cell lines. NaHS decreased the time required to respond to NMDA in a dose-dependent manner.

These studies provides evidence that H_2S, acting as a neuromodulator, facilitates LTP that is known to be involved with associated learning, and that it may modulate the synaptic activities regulated by steroid hormones and neurotransmitters. The authors suggest that the depressive effect of H_2S on synaptic transmission in the CNS may be partly responsible for the dizziness and unconsciousness following acute sublethal H_2S exposure. These findings support the reports by Kilburn and others (Tvedt et al, 1991b; Schneider et al., 1998) that have documented memory problems and increases in postural sway following H_2S exposure (Kuo et al. 1996; Hirasawa, 1976). Altered memory and learning was also reported in animal studies by Partlo et al. (1998) and Struve et al. (2001).

2.10.2.3 Neurochemistry

Neurons have a high metabolic rate that requires a continuous and abundant supply of oxygen and glucose primarily for maintenance of the membrane potential (Marieb, 1995). This indicates that the brain is an organ requiring high energy and thus may be more sensitive to the effects of its deprivation. It has been known for many years that sulphide is a potent inhibitor of cytochrome c oxidase (Ki 0.5 μM at pH 7.48; 0.07 μM at pH 6.28) (Nicholls and Kim, 1982). This has been demonstrated recently in mammalian brain (IC50 0.13 μM) (Nicholson et al., 1998) and based on preliminary results, in the olfactory and respiratory epithelium (Dorman et al., 1999). An earlier study found that repeated exposures of mice to 100 ppm H_2S for 2 hr for 4 days produced significant cumulative decreases in activity in the brain (Savolainen et al., 1980) (See Table A7.10.2; Appendix I), however only a decrease trend was observed in rat brain at lower levels (1, 10, and 100 ppm H_2S for 8 hr/d, 5d/wk for 5 wk) (Khan et al., 1998).

2.10.2.4 Neural Cell Growth

Pregnant rats exposed to 20 or 50 ppm H_2S for 7 h/day from day 5 post-coitus until day 21 postnatal had altered Purkinje cell structure (Hannah and Roth, 1991). The 50-ppm exposure resulted in significantly longer mean segment lengths whereas the 20-ppm exposure produced less dramatic results. Also, both H_2S exposures produced a left shift in the distribution of equivalent orders. There was a tendency toward smaller or a reduced equivalence in number of segments and splits from the root vertex.

2.10.3 Effects on Nervous System Enzymes and Neurotransmitters

Several investigators have reported significant alterations in neurotransmitters following H_2S exposure (Tables 18 and 19). No effects were found on regional brain catecholamine concentrations after rats were exposed to 0, 10, 30 and 80 ppm H_2S by whole-body inhalation for 3 hrs/day for 5 consecutive days (Struve et al., 2001). This contrasts with earlier findings of altered catecholamines in exposed rats (Skrajny et al., 1992; Warenycia et al., 1990; Warenycia et al., 1989a; Hannah et al., 1989; Kombian et al., 1989; Kombian et al., 1988) (See Table A20; Appendix I).

Timed-pregnant rats were exposed to 20 and 75 ppm H_2S for 7h/day from day 5 post-coitus to day 21 postnatal after which levels of serotonin and norepinephrine in the developing rat cerebellum and frontal cortex was determined (Skrajny et al., 1992). Serotonin and norepinephrine levels were significantly increased in the developing rats cerebellum and cerebral cortex. For the 75-ppm exposure, at day 14 and 21 postnatally, serotonin was increased in both brain regions. At days 7, 14, and 21 postnatally, norepinephrine was significantly increased in the cerebellum and at day 21 in the frontal cortex. Exposure to 20 ppm H_2S significantly increased the frontal cortex serotonin levels at day 21, and cerebellum and frontal cortex norepinephrine levels were significantly reduced at day 14.

Monoamine oxidase activity in brain tissue was measured after NaHS-poisoned brain tissue was treated with dithiothreitol to recover non-acid labile forms of sulfide (Warenycia et al., 1990). Recovery of non-acid labile forms of sulfide using dithiothreitol was greater than conventional acid hydrolysis. Monoamine oxidase enzyme activity was not only restored with dithiothreitol, but was increased 130 - 140% of baseline levels.

It should be noted that three copper[20] enzymes, dopamine ß-hydroxylase (DBH), monoamine oxidase (MAO) and peptidylglycine-amidating monooxygenase (PAM), play key roles in the production and metabolism of hormones, neurotransmitters and growth factors. MAO, located on the outer mitochondrial membrane, catalyzes the deamination of biogenic amines such as dopamine, noradrenaline, adrenaline, tyramine, serotonin, histamine and others in the brain to their corresponding aldehydes generating hydrogen peroxide in the process. It has a wide substrate specificity being able to oxidize primary, secondary, and tertiary amines. Inhibition of MAO by sulfhydryl reagents was first observed in 1945. Of the nine cysteine residues present in both MAOs, only two are important in MAO-A catalysis and three in MAO-B (Wu et al., 1993), as a redox-active disulfide at the catalytic center (Sablin and Ramsay, 1998). Agents that block DBH lead to a reduction of both norepinephrine and epinephrine in the brain whereas inhibitors of MAO cause an elevation of norepinephrine, dopamine and epinephrine (Cooper et al., 1996, p 290). These opposing effects may explain the variable findings of neurotransmitter levels following exposure.

Acute treatment with sulfide inhibits acetylcholinesterase (Manackjee, 1985; [cited in Reiffenstein et al., 1992]). However, cattle exposed to the Lodgepole blowout emissions were found to have increased activity in erythrocytes compared to controls and to subsequent samples taken one year later (Florence, 1996).

2.10.4 Gaps and Limitations

H_2S exposure can result in a variety of effects on the nervous system. Few of the human studies obtained good exposure data that could be clearly associated with the reported effects. Many diverse neurological effects are reported across a wide concentration range. One of the most reliable sets of H_2S measurements were likely those made by staff of Texas' regulatory agency in their air monitoring van (TNRCC, 1998). Symptoms of headaches and nausea reported by the staff were associated with median H_2S concentrations measured at 90 ppb for 5 hours downwind of a refinery. It would be useful to have better information on the distribution of measured concentrations. It still remains controversial whether very low levels result in neurological effects, yet in communities with geothermal activity with H_2S levels in the 'low ppb range' (most hourly measurements were less than 1 ppb or were not detectable and of 29 incident reports, a peak concentration of 302 ppb was measured) nervous system symptoms were reported 12 times more frequently than a control community.

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[20] Recall that the solubility product constant for CuS is very low (reported values of 10^-36 to 10^-38) and for Cu_2S is lower (10^-48). This indicates that, when both ions are present, very little of each are needed for complex formation. Also, this indicates that the complex does not readily dissociate once formed.

To address these gaps, efforts should be made to undertake symptom surveys that collect simultaneous site- or location- specific air monitoring data around sources such as landfills, pulp and paper processing plants, and sour gas processing facilities such as flares, batteries or other processing equipment. Additional studies should be undertaken to assess the effects of low levels of H_2S on brain chemistry, physiology and function. Future studies could address the following areas: a) effects on biochemistry of lipids, copper-containing enzymes (cytochrome c oxidase, MAO, DBH, PAM), neurotransmitters, energy production, and cell signaling molecules such as cAMP; b) physiological or functional effects on behavior, mood, motor function, and senses; and c) effects of H_2S on neural growth and development, particularly on neural cell adhesion molecules[21].

2.11 ODOUR

Chemical senses such as odour detection have developed in the most ancient animals to detect food, danger or potential mates. For humans, odour in outdoor and indoor environments has often been considered an aesthetic concern. Recent literature suggests that odours have more than aesthetic qualities, and may actually affect health, be a contributing factor in determining health or causing physical illness (Overcash et al., 1983; Ackerman, 1990).

Odor, smoke and noise, in rank order, are the three most frequent air quality complaints received by Alberta Energy and Utilities Board (1998, 2000). The board receives about 300 odour complaints from throughout the province annually, however the number of complaints has increased steadily for the past 4 years exceeding 400 in 1999 - 2000. For the last three years, the complaint/emergency phone line at Alberta Environment has received 1153, 1049 and 986 (as of Dec 3, 2001), public odor calls respectively, per year[22]. Since they began collecting information on June 6, 2001 to Dec 3, 2001, the Agriculture Food and Rural Development has received 190 odour complaints predominately from one facility[23].

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[21] These proteins are made by nearly all neural cells and are believed to promote non-specific adhesion involved in tissue assembly that begins with some of the earliest events in neural tube formation (Goodman, 1998). Some of the proteins in this family are P_0, myelin associated glycoprotein, N-CAM, fasciclin II, TAG-1, and L1.

[22] Personal communication Dec 3, 2001 with N. Welsh at the Environment complaint/Emergency toll-free line 1 800 222 6514.

[23] Personal communication Dec. 3, 2001 with M. Davison, at the Livestock Expansion and Development Unit, Alberta Agriculture Food and Rural Development.

Table 18 Nervous System Effects: Human Studies

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (OR 12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Thorn and Kerekes (2001) Literature review of health effects of sewage treatment plant workers	Fatigue and headache have been reported in several investigations.
Wing and Wolf (2000) Residents of 3 rural communities (one near an intensive hog operation, one near two intensive cattle operations and a third without livestock operations that use liquid waste management systems) were surveyed to assess health symptom and quality of life indicators.	Residents living near hog operations reported episodes of headache 7.6 times and blurred vision 1.25 times more than the reference community. Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community. Residents living near cattle operations reported episodes of headaches 1.57 times more than the reference community.
Vrijheid (2000) Literature review of health effects of residents living near hazardous waste landfills.	An increased prevalence of self-reported health symptoms such as fatigue, sleepiness and headaches among residents near waste sites was consistently reported in more than 10 of the reviewed papers.
Hessel & Melenka (1999) Case report of a 50-yr old oilrig worker with a history of 2 knockdowns (1981, 1988). This worker had no history of asthma or allergic disorders and was a lifetime non-smoker.	Chest tightness and shortness of breath that were made worse by exposure to chemical fumes, cigarette smoke and other irritants was reported. The worker reported that after the 1988 episode, he has persistent headaches and has noticed a reduction in his cognitive ability, power to concentrate, and has become more quick-tempered and irritable. A full neurological examination including cranial nerves, visual fields, muscle tone, deep tendon reflexes and a sensory examination was negative. A psychological examination revealed a man with a somewhat irritable disposition. His attempt at serial sevens was accurate but extremely slow, even with prompting and his reproduction of a 3-dimensional box was limited and his copying technique was poor. Lung function tests suggested an obstructive pattern. Bronchoscopy showed inflamed and friable airway mucosa.

Kilburn (1999) Five groups of exposed citizens or workers (total of 119) were evaluated by questionnaire and neurophysiological (battery of 28 tests) and psychological testing following exposure to H_2S and compared to 357 controls from areas free of known chemical contamination.	When exposed subjects were grouped according to best estimates of low, medium and high concentration (peak) exposures, an increased frequency of abnormalities was found in both the neurophysiological and neuropsychological tests compared to controls. In the lowest exposure group (H_2S ranged from 0.1 - 1 ppm with occasional peaks of 5 ppm), abnormal balance, color discrimination, grip strength and verbal recall were found. In the group exposed to the highest levels of H_2S (for 4 in group, range estimate of 0.3 - 30 ppm; for 7 in group that were rendered unconscious, range estimate of > 250 ppm to > 10,000 from personal monitor reading), abnormal results for choice reaction time, balance, color discrimination, visual fields, digit symbol, vocabulary, hearing, blink, grip strength, trailmaking, long term memory, culture fair and block design were found.
Boev et al. (1998). The physiological and biochemical states of children and adults who resided in the vicinity of the sulphide-containing gas processing plant were studied.	The chemical agents emitted by the plant were found to have adverse effects of the children's functional status, namely, decreased vital capacity of the lung, mental performance, retarded sensory-motor responses, altered enzymatic system activity.
Bates et al. (1998) Cancer registry and hospital discharge data to compare rates of cancer and disease in Rotorua, New Zealand with the rest of New Zealand. Rotorua sits on a geothermal field that has continuous ambient low-level H_2S (median conc. 20 ug/m3; 14 ppb) and mercury.	Standardized Incidence Ratios (SIR) for diseases of the nervous system and sense organs were increased (SIR 1.11; 95%CI 1.07 - 1.15). These were further classified and increases were found for other disorders of the CNS (SIR 1.22; 95%CI 1.11 - 1.33); disorders of the peripheral nervous system (SIR 1.35; 95% CI 1.21 - 1.51). Statistically significant increased were also found for infantile cerebral palsy (SIR 1.42; 95%CI 1.03 - 1.89); migraine, (SIR 1.40; 95% CI 1.12 - 1.72); other brain conditions (SIR 2.50; 95% 1.89 - 3.26); mononeuritis of upper limb and mononeuritis multiplex (SIR 1.47; 95%CI 1.29 - 1.67); and mononeuritis of lower limb (SIR 2.06; 1.46 - 2.81).
DeFruyt et al. (1998) 187 viscose rayon workers in Belgium underwent an extensive neuropsychological examination as a part of an extensive health survey. 67 served as non-exposed controls. 120 were, for at least a year, exposed to CS_2 and H_2S. The CS_2 concentrations ranged from 3 - 147 mg/m^3 (1 - 47 ppm) and H_2S were below 14 mg/m^3 (10 ppm).	The neuropsychological investigation included subtests of the Weschler Adult Intelligence Scale, the entire Wechsler Memory Scale, the Bourdon-Wiersma Test, the Santa Ana Dexterity Test, the Gibson Spiral Maze, and the Bimanual Sinusoidal Movement Test. Only the group exposed to values exceeding three times the recommended limit for CS_2 (31 mg/m^3; 10 ppm) had significant impairments in both the speed and quality of psychomotor performance. Exposure to CS_2 and H_2S had no significant effect on memory and attention.

Schneider et al. (1998); Anonymous (1993) Case report of the long-term effects of H_2S exposure in a previously healthy 27-year old male construction worker. The man was popular, well-adjusted, conscientious student who performed above grade level on academic indices. The man was part of a crew that was building a sewer system in a New Jersey wetland. The man was exposed to H_2S as he descended a ladder into the 27-foot-deep pit to rescue a co-worker. He fell an unknown distance but there was no evidence of head trauma (skin abrasions, bruising, normal head CT). Measurements at the site four hours after the event indicated concentrations of 22 ppm H_2S at the surface of the water.	This worker was rescued and admitted to hospital with a Glasgow Coma Score of 3, pupils dilated and sluggish and several cardiovascular abnormalities. At the scene of the accident, the worker reportedly had seizures and in the emergency room, seizure activity and movements similar to those observed following removal of the brain or cutting the spinal cord at the level of the brainstem were described. Corneal reflexes were absent and deep tendon reflexes were 2/4. Five days after admission, after receiving various interventions, the worker regained consciousness, could respond to simple commands, and began to feed himself and move around with assistance. However he was agitated, restless and had impaired language, memory and attention. Sixteen days after hospital admission, he entered a rehabilitation facility and was found to have slowed speech, impaired attention span, retrograde amnesia with confabulation, decreased insight and ability to communicate, flat affect, and impaired visual memory with poor acquisition, retention and recall of new information. After about 1 month, leaving the rehab unit, neuropsychological evaluation revealed difficulty on verbal and visual memory tasks and associative learning. There were also left-sided deficits in balance and speed of movement. Over the 4 years since the exposure, family and health professionals note that the man has continued problems with short-term memory, sequential thinking, decreased attention and lack of initiative. Three years after, a positron emission tomography scan showed markedly decreased metabolism in the left thalamus, heterogeneous and abnormal uptake in the basal ganglia, and abnormally decreased metabolism in both temporal and inferior parietal lobes. A cerebral perfusion study done 3.5 years later showed bilaterally decreased activity in the putamen and the amygdala/hippocampal region. Neuropsychological and neurofunctional testing showed abnormally small body size, psychomotor slowing, extrapyramidal signs and deficits in memory, executing, planning and functioning.
Kilburn (1998) Neurophysiological and psychological testing was done on five groups and were compared to age- and education-matched controls: a) 16 H_2S -exposed workers, b) 68 subjects affected by a refinery explosion and gas leak (Torrence), c) 13 former Unocal workers and 22 downwind residents of Unocal refinery, d) residents of two communities with oil refineries and e) refinery workers evaluated for asbestos exposure.	In group a), compared to controls, abnormal test results were found for: balance, choice reaction time, simple reaction time, blink reflex latency, color discrimination, visual fields, vibration sense, fingertip writing, trail making, culture fair, block design, vocabulary, immediate verbal recall, visual reproduction and profile of mood state. Group b) had abnormal test results for simple and choice reaction times, color vision, vocabulary, trailmaking, and profile of mood states. Group c) had abnormal test results for simple and choice reaction time, balance, color vision, trail making, immediate story recall, and digit symbol. In group d) abnormal test results were found for choice reaction time, and balance. In group e) abnormal test results were found for balance.

TNRCC (1998) Six workers were exposed to a mean concentration of 0.09 ppm H_2S for approximately 5 hours in a monitoring van downwind from an oil refinery	Persistent odors, eye and throat irritation, headache and nausea were observed in the workers.
Kilburn (1997) Neurophysiologic (simple reaction time, visual two-choice reaction time, body balance, blink reflex, color discrimination, visual fields, and vibrations) and psychologic tests (Wechsler's memory scale, Culture Fair battery, Wechsler Adult Intelligence Scale, Halstead-Reitan battery, Multidimensional Aptitude battery, Profile of Mood States) were used to appraise mood status and symptom frequencies in 16 H_2S -exposed subjects, categorized into minutes-, hours- and years-exposure groups, 2 - 22 years after exposure. The exposed individuals also completed a self-administered questionnaire. The results were adjusted for age, sex, educational achievement and other factors and compared to 353 unexposed referents.	Symptom frequencies as group means in eight categories of mucous irritation, chest symptoms, sleep, memory, balance, mood, gastrointestinal symptoms, and limbic functions were elevated significantly above the unexposed group. Balance with eyes closed was impaired in 75% of H_2S -exposed subjects with their mean sway speed 2.5 times greater than controls. Balance with eyes open was impaired in 56% of patients, with the mean sway speed 59% higher than controls. Choice reaction time was prolonged in 63% of patients and the mean of the group was 30% greater than controls. 43% of H_2S -exposed subjects had prolonged simple reaction times, 45% had slowed blink reflex latency, and color discrimination and visual fields were abnormal in 83 and 77%, respectively. Vibration sense was reduced in 91% of subjects, and hearing acuity was reduced 60 - 74%. Scores on cognitive domain tests (Culture Fair, block design, digit symbol, and vocabulary) were all significantly below controls. Peg placement and trail making were decreased; verbal recall was decreased and scores on memory tests were decreased significantly. The profile of mood states score was elevated in 63% of patients.
Anonymous (1996) Air testing was done near 17 large-scale hog manure lagoons, some as large as a city block, in Renville County by Land Stewardship Project members and staff.	Area residents complained of nausea, headaches, blackout periods, vomiting and other symptoms. During a 2-week period in May, a Jerome Analyzer was used to measure H_2S concentrations near the lagoons as well as up to 1.5 miles away. All of the sites showed some H_2S in the air. Two of the tests showed mean H_2S levels of more than 100 ppb, with one site measuring 134 ppb. Eight of 32 tests showed mean H_2S concentrations of more than 50 ppb.
Berger (1996) [cited in McGavran, 2001] A study of self-reported symptoms among residents living in an area adjacent to and lower than a landfill in Florida was done.	Levels of H_2S as high as 782 ppm were detected above the landfill. Air escaping the landfill in pockets had levels from 20 - 128 ppm and levels greater than 100 ppm were consistently found. Headache and eye irritation were the most commonly reported symptoms. Respiratory infection, nasal and throat irritation were also reported.

Haahtela et al. (1992) Subjects living downwind of a paper mill that released H_2S and mesityloxide for two days, The 24-hour average H_2S concentration for the two days was 35 and 43 ug/m3; a 4-hour maximum was 135 ug/m3; mesityloxide was not measured.	Significantly increased symptoms of difficulty breathing in 33% and increased mental symptoms (depression and anxiety) in 10% of 75 subjects living downwind of a paper mill. Sixty-three percent of respondents reported at least one symptom following the release compared to 26% during the reference period, 4 months later during a low-exposure period. Eye symptoms, cough or pharyngeal irritation, breathlessness, nausea and headache were experienced more often during the release period than the reference period.
Partti-Pellinen et al. (1996) Cross-sectional study of 336 adults living near a pulp mill and of 380 adults in a reference community for both 4-week and 12-month prior periods. In the exposed community, the annual mean TRS and SO_2 concentrations measured were 2-3 ug/m3 and 1 ug/m3, respectively. In the reference community, the SO_2 concentration was 1 ug/m3.	After controlling for confounders, compared to the reference community, increased risks for headache or migraine in the previous 4 weeks (OR 1.83; 95% CI 1.06 - 31.5) or past 12 months (OR 1.70; 95% CI 1.05 - 2.73).
Kuo et al. (1996) Postural stability was measured in 28 sewer workers who have worked at the plant at least 6 months. Volatile organic solvent concentrations, measured with a photoionization detector as benzene equivalents were also measured.	The mean (+/- SD) solvent concentration was 0.32 +/- 0.19 ppm benzene equivalent; range 0.02 - 0.95ppm. Compared with a non-exposed population, the workers had increased postural sway. Statistically significant positive correlation was found between postural sway (r = 0.91 for sway length; 0.72 for sway area) and organic solvent exposure. Yet the solvent exposure was very low and the authors suspected that some other agent, rather than the solvents might be responsible.
Pach et al. (1996) Report of investigation of health of inhabitants living near a large refuse dump.	Elevated methemoglobin levels were found in 8 patients. Increased blood lactate was found in 14 people and elevated levels of carboxyhemoglobin were found in 8 people. Gases identified from the dump were carbon dioxide, methane, carbon monoxide, hydrogen sulphide, methane homologues and aromatic hydrocarbons. Typical symptoms of methemoglobinemia were not observed however, frequent headaches and neurovegetative disorders were found.
Snyder et al. (1995) Case report of an incident where 37 people were exposed to H_2S emanating from a pit dug into a coastal wetland. H_2S and methane were measured however actual concentrations were not given.	At least one victim, who underwent extensive treatment, developed persistent neurological sequelae. These included slow speech, flat affect, moderately impaired attention span, easy distractibility, isolated retrograde amnesia, decreased insight and ability to communicate, markedly impaired visual memory with poor acquisition, retention and recall of new information. These findings were unchanged at 12 and 18 months after exposure.

Kilburn and Warsaw (1995) Six domains of brain function (neuro-physiological, recall, overlearned memory, cognitive, perceptual motor speed and affective) of 13 former workers (none had been overcome by H_2S) and 22 neighbors of a sour crude oil refinery living within 1200 meters of the plant were compared to 32 age-, gender- and education-matched controls. A questionnaire was administered to assess frequency of irritative, respiratory and neurological symptoms. Worker exposures were not measured.	The exposed subjects' mean values were significantly different from controls for two-choice reaction time, balance (as speed of sway), color discrimination, digit symbol, trail-making A and B, and immediate story recall. Visual recall was significantly impaired in neighbors but not in the ex-worker group. Anger, confusion, depression, tension-anxiety and fatigue scores were significantly elevated in neighbors and former worker groups compared to controls. Respiratory, mucous membrane irritation, neurological symptoms, sleep disturbances, and skin complaints were all more frequent in exposed subjects, compared to controls. Excluding subjects with other chemical exposures (surgical anesthesia, solvents, vibrating tools, seizure medication, pesticide, alcohol) did not affect the results significantly. H_2S, measured at street level for 1 week, was 10 ppb with periodic peaks of 100 ppb. Dimethylsulfide was 4 ppb, mercaptans were 2 ppb, and ethane was 500 ppb and propane, 500 ppb. 24-hr averages of gases monitored outside the desulphurization unit were 0.1 - 21.1 ppm mercaptans, 0 - 8.8 ppm H_2S, 2.6 - 52.1 ppm COS, and 6.1 - 70.1 ug/m3 total reduced sulphur.
Anonymous (1995) Investigators of the National Institute for Occupational Safety and Health conducted a health hazard evaluation at a wastewater treatment plant in Independence, Missouri.	Employees reported fatigue and headaches and other complaints while working in the belt pressroom. Measurements of personal breathing zone concentrations of H_2S were: maximum 10-minute ranged from 0.1 ppm to 95 ppm; 8 of the 13 personal breathing zone samples exceeded 10 ppm and 3 exceeded 20 ppm. Maximum 10-min concentrations in general air samples obtained in the belt pressroom range from 46 - 69 ppm whereas outside the belt pressroom, the maximum 10-min concentrations ranged from non-detectable to 0.1 ppm.
Schiffman et al. (1995) The effect of environmental odors emanating from a large hog operation on the mood of 44 residents living nearby was determined using the Profile of Mood States (POMS). They were compared to 44 control subjects who were matched according to gender, ethnic group, age and years of education.	A significant difference between control and experimental subjects for all six POMS factors and the total mood disturbance score was found. People living closest to the intensive swine operation who experienced the odors had significantly more tension, more depression, more anger, less vigor, more fatigue, and more confusion than control subjects. People exposed to the odors also had greater total mood disturbance than controls as determined by their ratings on the POMS.
Marttila et al. (1994b) The effects of long-term exposure to malodorous sulphur compounds on 134 children's respiratory health were assessed by administering questionnaires and comparing the occurrence of symptoms in variously polluted areas.	The estimated and measured concentrations of malodorous sulphur compounds are in Table 7.14.2. Compared to the non-polluted area, for exposures to severely polluted air during the previous 12 months, increased headache [OR 1.77; CI 0.69 - 4.54] was reported.

Sanz-Gallen et al. (1994) Case report of three workers exposed to emissions for 50 - 60 minutes inside a cellulose tank.	All three workers lost consciousness; one developed a persistent vegetative state; another recovered although with neurological effects; the third patient completely recovered one week after hospitalization.
Arentoft et al. (1993) Case report of hydrogen sulphide intoxication in a farmer who collapsed inside a mobile tank, recently used for manure spreading. On-site measurements showed high concentrations (values not given).	The farmer arrived at the hospital in a coma, survived, but suffers from a toxic/anoxic psychosyndrome.
Callender et al. (1993) Workers exposed to H_2S and other substances that developed clinical encephalopathy after exposure were studied by SPECT brain scans. Neuropsychological testing and other diagnostic procedures and their results are described.	7 of the 9 workers exposed to H_2S (and in all cases, other substances) had abnormal SPECT neuroimaging results. The scans of the cerebellum were abnormal for 2/9 workers; the frontal lobe 4/9, temporal lobe 3/9; thalamus, 1/9; cerebrum 1/9 and basal ganglia 5/9 workers. Neuropsychological testing was abnormal in 4/6 exposed workers, with deficits occurring the attention and mental flexibility (3/6), learning and memory (2/6), motor (2/6) and visual-spatial (1/6). Neurophysiological testing showed abnormalities of color in 6/7 workers; of odor in 5/8; and of current perception threshold in 7/9.
Inoue (1993) Literature review of Parkinsonism among toxic diseases	Parkinsonism has been described following H_2S exposure.
Haahtela et al. (1992) Subjects living downwind of a paper mill that released H_2S and mesityloxide for two days, The 24-hr average H_2S concentration for the two days was 35 and 43 ug/m3 (25 ppb, 30 ppb); a 4-hr maximum was 135 ug/m3; mesityloxide was not measured.	Significantly increased symptoms of increased mental symptoms (depression and anxiety) were found in 10% of 75 subjects living downwind of a paper mill. Sixty-three percent of respondents reported at least one symptom following the release compared to 26% during the reference period, 4 months later during a low-exposure period. Eye symptoms, cough or pharyngeal irritation, breathlessness, nausea and headache were experienced more often during the release period than the reference period.
Hua et al. (1992) Case report of young man exposed to H_2S	Abnormal neurological effects of a young man exposed to H_2S were found. These included: inability to express oneself in writing, impairment in the ability to perform arithmetic operations, deficits in associative verbal fluency and design fluency, and general intellectual deterioration.

Tvedt et al. (1991b) Case report of a formerly healthy shipyard worker rendered unconscious after exposure to H_2S while overhauling an oil-drilling platform.	In addition to severe immediate neurologic signs and symptoms, follow-up 5 years later showed slight cerebral atrophy by MRI and CT scans, motor (difficulty walking stairs), memory (no recollection of events of accident) and visual symptoms (air appeared to vibrate), reduced understanding of speech, migraine headaches, and unable to resume work. Neuropsychological testing showed deficits in motor function and memory tests. Restrictive visual fields, disturbed vision, and hearing loss that resolved were also reported. This worker had also become sensitive to strong smells.
Jappinen et al. (1990) Respiratory function was assessed on 26 male pulp and paper workers before and after exposure to H_2S concentrations usually below 10 ppm. Similarly, respiratory function of 10 asthmatic volunteers was assessed before and after exposure to 2 ppm H_2S for 30 min.	Asthmatic subjects exposed to 2 ppm rapidly adjusted to the smell of the gas however three of them (30%) complained of headaches.
Shusterman et al. (1989) The sulphur gases were below the detection limit of sensitive air monitoring equipment (0.1 ppb for H_2S; 0.02 ppb for mercaptans).	Increased frequency of headaches, upper respiratory tract irritation, and nausea among community members downwind of a hazardous waste site following the release of sulphur gases from settling ponds.

Table 19 Nervous System Effects: Animal Studies

Reference / Study Design	Key Findings
Struve et al. (2001) Adult male CD rats were exposed to nose-only inhalation of 0, 30, 80,200 or 400 ppm H_2S or to whole body inhalation (0, 10, 30, 80 ppm H_2S) for 3 hr/day for five consecutive days. The nose-only group was tested immediately after exposure in a Morris water maze. The whole-body inhalation group was tested for spontaneous motor activity immediately following the fifth exposure. Catecholamine levels in the striatal, hippocampus and hindbrain were determined.	Significant reductions in motor activity, water maze performance and body temperature was found following exposure to only high H_2S concentrations (> or = 80 ppm). H_2S exposure did not affect regional brain catecholamine concentrations or performance on the multiple fixed interval schedule.
Dorman et al (2000) Rats were exposed to H_2S at various concentrations (0, 10, 30, 80 ppm; 6 h/day; 7 days/wk) for 2 weeks prior to breeding, for a 2-wk mating period, and then from gestation day 0 (gestation day 0 = evidence of copulation) through day 19. Exposure of dams and their pups resumed between postnatal day 5 and 18. Adult male rats were exposed for 70 consecutive days. The animals were subjected to various neurological tests (motor activity, passive avoidance, functional observational battery, acoustic startle, neuropathology), at various times during the exposure period. At the end of exposure period, the animals were sacrificed and their tissues examined.	There were no deaths and no adverse physical signs during the study, nor any statistically significant effects on reproductive performance. Exposure to H_2S, reportedly did not affect pup growth, development, or performance on any of the behavioral tests.
Partlo et al. (1998) Rats were repeatedly exposed to 125 ppm H_2S (4 hr/day, 5 days/week for 5 weeks), their ability to learn and relearn new tasks was evaluated with a 16-arm maze.	Repeated exposures interfered with learning new tasks and relearning tasks.
Khan et al. (1998) Rats were repeatedly exposed to 0, 1, 10, and 100 ppm H_2S for 8 hr/d, 5d/wk for 5 wk to study the effects of exposure on various enzyme activities in red blood cells, lungs, liver and brain tissue.	No changes in enzyme activities were found for the 1-ppm exposure regimen. Significantly lower activities of lung mitochondrial cytochrome c oxidase were observed in animals exposed to 10 and 100 ppm H_2S. In mitochondria isolated from brain, there was a trend toward decreased cytochrome c oxidase activity as the H_2S concentration increased from 1 to 10 and to 100, however this was not statistically significant.

Stair et al. (1996) The health of 460 beef cattle and their calves within 3 miles was assessed after a pipeline leak of volatile components of crude sour petroleum, emissions from burning sour condensate and steam washing of gravel occurred at the Red Deer River.	Clinical observations included irritation of mucous membranes, abnormal sexual behavior, decreased bonding of cows with newborn calves and failure of calves to thrive. Some cows had proprioceptive-locomotor deficits attributed to the central nervous system and an increased rate of twinning was present. Histology showed possible sensory neuronal hypoplasia in the dorsal horns of the spinal gray matter in stillborns and neonates; gray matter at junction with white matter in dorsal horn of spinal cord in neonatal calf is essentially without neurons; axonal degeneration in the peripheral nerves within the Gasserina ganglion trigeminal nerve; cytoplasmic neuronal vacuolation and amphicytic proliferation around ganglion cells.
Baldelli et al. (1993) Ventilated and unventilated rats were injected intraperitoneally with sodium sulfide to determine its effects on brain function and morphology and physiological parameters such as blood pressure, EEG, arterial blood gases and pH.	Compared to unventilated rats, mechanical ventilation reduced the amount of sodium sulfide required to kill 50% of exposed animals by about 2-fold. Except for one animal with prolonged hypotension, cerebral necrosis was not found in the ventilated rats.
Sandu et al. (1993); [cited in Gangolli, 1999]	H_2S inhibited the oxidation-energy metabolism and membrane transport processes in the brain regions of males rats exposed to H_2S
Boev et al. (1992) Rats were exposed to gas condensate containing H_2S.	Disturbance of elaboration and reproduction of conditioned reflex of *two-way avoidance was found. Accumulations of lipid peroxidation* products were found in the cerebral cortex. Catalase was inhibited.
Skrajny et al. (1992) Timed-pregnant rats were exposed to 20 and 75 ppm H_2S for 7h/day from day 5 post-coitus to day 21 postnatal. Levels of serotonin and norepinephrine in the developing rat cerebellum and frontal cortex were determined.	Serotonin and norepinephrine levels were significantly increased in the developing rats cerebellum and cerebral cortex. For the 75-ppm exposure, at days 14 and 21 postnatally, serotonin was increased in both brain regions. At days 7, 14, and 21 postnatally, norepinephrine was significantly increased in the cerebellum and at day 21 in the frontal cortex. Exposure to 20 ppm H_2S significantly increased the frontal cortex serotonin levels at day 21, and cerebellum and frontal cortex norepinephrine levels were significantly reduced at days 14. Since monoamines influence neural development, cell division, migration, morphogenesis and synapse formation, it was concluded that H_2S may cause irreversible changes in the developing central nervous system.

Hannah and Roth (1991) Pregnant rats were exposed to 20 or 50 ppm H_2S for 7 h/day from day 5 post-coitus until day 21 postnatal. The pups' brain tissue was removed and processed using Sholl's Golgi-Cox method, and was embedded in celloidin and sectioned at 100 um. Purkinje cells were analyzed by a digitizer to count the number of dendritic trees on the basis of nodal vertex types and to measure the branch length at each division.	The 50-ppm exposure resulted in significantly longer mean segment lengths whereas the 20-ppm exposure produced less dramatic results. Also, both H_2S exposures produced a left shift in the distribution of equivalent orders. There was a tendency toward smaller or a reduced equivalence in number of segments and splits from the root vertex. These changes, when extrapolated to the entire dendritic field, indicate severe effects to purkinje cell dendritic fields architecture and growth.
Warenycia et al. (1990) NaHS-poisoned brain tissue was treated with dithiothreitol to recover non-acid labile forms of sulfide. Monoamine oxidase activity in brain tissue was measured.	Recovery of non-acid labile forms of sulfide using dithiothreitol was greater than conventional acid hydrolysis. Monoamine oxidase enzyme activity was restored with dithiothreitol, and was increased 130 - 140% of baseline levels.
Hannah et al. (1990) Timed-pregnant rats were exposed to 50 ppm H_2S for 7 hr/day from day 6 post-coitus until Day 21 postnatal in an environmental chamber. Taurine levels were monitored on day of parturition and day 21 postnatal.	Maternal blood taurine levels were significantly greater (30%) than controls at both sample times.

Table 20 *In vitro* **Neurochemical Effects**

Reference / Study Design	Key Findings
Kimura (2000) Primary cultures of rat brain cells (neural and glial) were induced by NaHS to produce cAMP in the presence and absence of inhibitors. The effect of H_2S on expression of NMDA receptors was also tested.	Physiological concentrations of NaHS induced cAMP production in primary cultures of brain cells, neuronal and glial cell lines, and *Xenopus* oocytes in a dose-dependent manner. NaHS decreased the time required to respond to NMDA in a dose-dependent manner. However, not all neural cells lines responded to the same degree.
Abe and Kimura (1996) mRNA expression of cystathionine beta synthase (CBS) and cystathionine gamma lyase was evaluated by Northern blot analysis in rat hippocampus, cerebellum, cerebral cortex and brainstem. H_2S production was measured in brain homogenates in the presence of activators and inhibitors. The effect of NaHS on synaptic transmission, on long term potentiation (LTP), and NMDA receptor-mediated responses was also measured in the CAI region of rat hippocampal slices	CBS was highly expressed in the hippocampus and cerebellum and less so in the cerebral cortex and brainstem. Brain homogenates produced 22.6 +/- 1.6 nmol H_2S / min per gram of protein in the presence of 10 mM cysteine and 2 mM pyridoxal 5'-phosphate. CBS inhibitors suppressed H_2S production in a concentration dependent manner. Low concentrations of NaHS (< 130 uM) did not affect the synaptic transmission in the hippocampus whereas higher concentrations (320 and 640 uM) suppressed both field potentials and population spikes. Occlusion experiments showed that H_2S -induced LTP shares common mechanisms with LTP induced by a strong tetanic stimulation. Using a NMDA receptor antagonist, 130 uM NaHS did not induce LTP suggesting that the induction requires activation of the NMDA receptor. NaHS was found to selectively enhance NMDA receptor mediated currents at physiological concentrations, which were not affected by dithiothreitol. The thiol redox sites were thought to contribute little to the potentiating effects of H_2S on the induction of LTP.
Kombian et al. (1993) The effects of sodium sulfide on neurons from *Rana pipiens* were evaluated.	The ability of the neuron to maintain normal electrochemical gradients was significantly affected, with 1.8 mM sodium sulfide producing a membrane depolarization of 2.75 eV, which was independent of extracellular Na concentration. Influx of Ca2+ was not evaluated and was suspected of being affected.
De Santis et al. (1990) The effects of methanethiol, dithiothreitol and N-ethyl-maleimide were studied on the electrical activities of the frog spinal cord.	Methanethiol depressed spontaneous dorsal and ventral root potentials and had no effect on evoked potentials. Dithiothreitol dramatically increased spontaneous electrical spinal cord activity pattern. Together the results suggest the presence of interneuronal membrane sulfhydryl groups of the dorsal horn that are involved in the origin of spontaneous electrical cord activities.

2.11.1 Physiology of Odour Recognition

For land animals, the initial step in olfactory response involves the interaction of an odour, usually a small hydrophobic volatile organic molecule, with specific receptors located on the surface of the olfactory sensory neuron (OSN). As bipolar cells, OSN dendritic ends are comprised of cilia, which extend into an unbranched myelinated axon, which penetrates the skull in the cranial cavity, and terminates in the olfactory bulb, at the base of the brain. After entering the nose, the odorants dissolve in the mucous lining, bind to specific receptors on the neuron's cilia, thereby triggering a cascade of signal transduction events involving G-protein-dependent elevation of cAMP, opening of ion channels and membrane depolarization (Lazard et al., 1989; Mombaerts, 1999). The odorant receptor gene family is the largest family in the mammalian genome, comprising about 0.8 - 1.6% of the 60,000 or so mammalian genes (Mombaerts, 1999).

The signals received at the olfactory bulb converge with relay neurons in a series of spherical neuropils called glomeruli before being sent off to higher forebrain centers. Olfactory information received at olfactory receptors is transmitted to several regions of the brain, both cortical and subcortical, affecting memory formation and retrieval, modulation of emotional responses via the limbic system, and regulation of neuroendocrine function such as the hypothalamus (Cone and Shusterman, 1991). Odorous compounds, even when presented at concentrations that cannot be consciously detected, produce distinct electroencephalogram responses as well as impairment of mood and performance (Schiffman, 1998). There are inextricable anatomical connections between the mammalian limbic and olfactory systems, and the connections have been demonstrated in conscious monkeys and humans.

Approximately 40% of the neurons in the rodent amygdala respond to olfactory stimulation (cited in Zald and Pardo, 1997). In rats, the basolateral complex receives and integrates sensory information from a variety of sources, acting as a locus of sensory convergence within the amygdala (Maren, 1999). Auditory, visual, somatic, spatial and olfactory signals converge and are conveyed to the central nucleus where projections to the hypothalamus and brainstem mediate fear responses, potentiate acoustic startle, increase heart rate and blood pressure, increase respiration and glucocorticoid release (Maren, 1999). In humans, Kettenmann et al. (1997) found consistent activation of the anterior-central parts of the insula, the parainsular cortex and the superior temporal sulcus following olfactory stimulation with two odorants, vanillin and hydrogen sulfide. Yousem et al. (1999) found that the right frontal lobe is highly associated with olfaction and that women activate significantly more areas (5 - 15-fold) of the frontal and temporal lobes of their brains than men presented with the same odorants.

Inhalation of odorants increases electrophysiological signaling in the medial amygdala (Zald and Pardo, 1997). Upon breathing a mixture of sulfides (25 ppm each of dimethylsulfide, ethanethiol, and methanethiol), 12 women found the mixture to be highly aversive and highly intense, to increase muscle tension, repulsion, disgust, or to increase fear that the gases were dangerous. These evaluations corresponded with changes in regional blood flow in the left amygdala that were not observed with fruity, spicy or floral scents. Functional magnetic resonance imaging has also shown lateral amygdala activity following unpleasant odors (Wexler et al., 1995). These findings provide support to the critical role of the human amygdala in either the processing of aversive olfactory stimuli or the transduction of neural signals from smells into emotional responses.

2.11.2 Olfaction and Respiration

Recent work suggests that in humans, the sensitivity of respiratory measures such as breath volume reductions may not lag far behind that of perceptual measures of irritation (Warren et al., 1994). In prior work, the minimum concentration required to lower breath volume was only about 1 log unit higher that the nasal irritation threshold. For acetic acid, amyl acetate, and phenyl ethyl alcohol, in 10 subjects, the volume of air inspired per breath appeared to be a reliable physiological correlate of perception, suggesting that the reduction in tidal volume may be a useful correlate of the point at which the trigeminal nerve begins to contribute to perceived nasal irritation.

2.11.3 Odour Thresholds

For an odorant to be sensed or smelled, its concentration must exceed some specific level, which is called a threshold concentration (Winneke, 1992). Olfactometers, devices that allow for precise dilution of chemicals and repeated presentation to panelists for detection, are used to determined thresholds for odorant chemicals.

Age has been found to strongly affect odour sensitivity. Olfactory sensitivity to H_2S declines in a linear fashion with age such that a 55- year-old has about 10-fold less sensitivity than a 20-year old (from figure cited in Winneke, 1992). Women tend to perform better than men across all age groups on standardized tests of odor detection and identification (Yousem et al., 1999).

People can detect a wide range of H_2S concentrations (Table 21). Hoshika et al. (1993) compared published odour threshold values for six odorants and found that there were striking similarities for H_2S odour thresholds between Japan (0.5 ppb) and the Netherlands (0.3 ppb) even though slightly different methods were used to determine 'the barely perceptible concentration".

It is apparent that 50% of the people tested were able to detect H_2S concentrations of about 5 ppb (range 3-10); 25% were able to detect concentrations of 2 -3 ppb. These values are consistent with those reported by Schiffman (2000) and Hoshika et al. (1993).

2.11.4 Factors Modulating Responses to Odours

Ackerman (1990) reported that odours could result in strong emotional and physical responses, particularly after repeated exposures. Schiffman (1998) has reviewed the literature that addresses modulating factors such as memory, mood, learned responses, and attitudes and perception, which are briefly summarized here. Odours can affect memory, prompting recall of early memories, and especially for women, emotionally laden memories. Unpleasant odors can alter mood and induce stress responses. Conversely, pleasant odors can improve emotional and physical health, improve mood, reduce stress and anxiety, increase alertness and performance,

Table 21 Odour Threshold Studies

Number of Test Subjects (Age)	H_2S (ppm)	Odour Effects	Reference
26 studies	0.0005 0.002 0.004 0.008 0.030	2 % of popl'n detecting 14 % 30 % 50 % 83 %	Schiffman (2000)
na	0.0003, 0.0005	Odour threshold in Japan and Netherlands, respectively	Hoshika et al. (1993)
na	0.0081	Odour threshold	Amoore and Hautala (1983)
na	0.00079 - 0.0017	na	Brunekreef (1980)§
na	0.053	Detection	Nishida (1979)§
na	0.0019	Detection	Winneke (1979)§
na	0.0011	Detection	Thiele (1979)§
na	0.019 0.19	Detection Odour threshold	Williams et al. (1977)
na	0.11	Threshold of objectionability (not odour)	Tonzitich and Ng (1976)
na	0.0010 - 0.039	na	Nishida (1975)§
na	0.048	na	Stephens (1971)§
na	0.0086	na	Randebrock (1971)§
na	0.012 - 0.069 [geometric mean was 0.029 +/- 0.005]	A perception study found the odour thresholds for 16 individuals exposed to increasing H_2S concentrations.	California State Dept. of Public Health (1969)
4 trained odour analysts	0.00047	Lowest concentration at which all subjects recognized the odour.	Leonardos et al. (1969)
na	0.0045	Median threshold of 33 observers (range 0.9 - 22 ppb).	Wilby (1969)
na	0.0034 - 0.0065	Range of 'calculated' odour thresholds within several groups	Adams et al. (1968)

na	0.0086	na	Basmaczhieva (1968)§
na	0.0057 - 0.0079	Detection	Young (1966)§
na	0.0072	Detection	Cederlof (1966)§
14 (18 - 30)	0.010 - 0.022	Range of odour thresholds within a 'practically healthy' group	Baikov (1963)
12	0.0086 - 0.022 (20 - 35 observations)	Range of minimum concentrations at which the odour was smelled	Duan (1959)
11 (16 - 55 yr)	0.008 - 0.018 0.07	<- odour not perceptible <- most perceived odour	Loginova (1957)

*na - not available

§ cited in American Industrial Hygiene Association (1989).

Anspaugh and Hahn (1980) summarized the odour perception threshold literature (Figure 1).

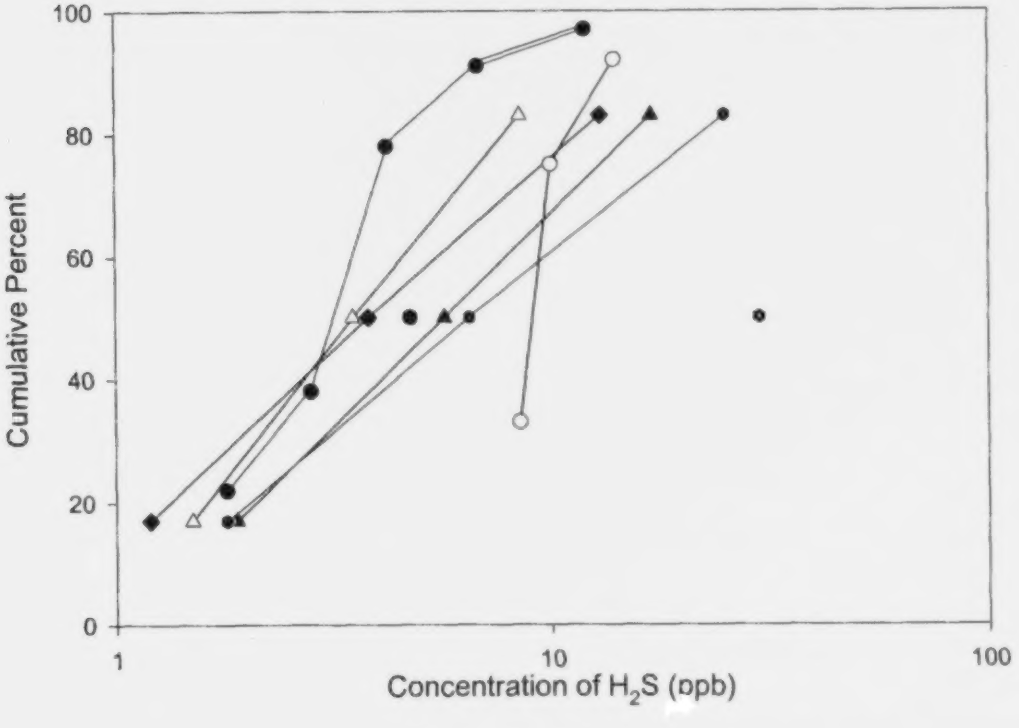

Figure 1 **Cumulative Percentage Distribution for H_2S Odor Detection (Adapted from Anspaugh and Hahn, 1980)**

benefit sleep patterns and improve immune status. These responses have been attributed to the anatomical overlap of the olfactory and limbic (emotional) systems in the brain.

Learned associations may influence odour-induced symptoms. As well, cognitive expectations or beliefs about the safety of an odour modulate an individual's perception in a small but significant manner. That is if people are prompted either negatively or positively before presentation of an odour, the responses are biased in the same direction as the prompt. Adaptation, the reduction in responsiveness, during or following repetitive exposure occurs on a short (minutes) or long-term basis (hours or days). Conversely, sensitization, the increased responsiveness during or following exposure has also been reported. Intermittent exposures to odours may also modulate responses perhaps by clonal expansion of high affinity compound-specific olfactory receptors. Reductions in symptoms have been reported with behavioral changes that included exercise, biofeedback, systemic desensitization and progressive muscle relaxation training to relieve stress. The perception of control has also been shown to reduce anxiety.

Complaints about aversive odors are strongly influenced by the modulators discussed above and involve a complex interaction between biology, behavior, physiology and psychosocial influences.

2.11.5 Adverse Effects of Odorants

There are substantial animal toxicology data demonstrating damage to the olfactory neuroepithelium and bulb by airborne chemicals (Cowart et al., 1997), as well as a large and scattered literature on the adverse effects on the sense of smell of occupational exposures to industrial chemicals (Amoore, 1986). Acute exposure to H_2S has been reported to cause permanent and temporary hyposmia (reduced sense of smell) (Amoore, 1986). Early studies reported that exposures of 110 ppm for seconds produced loss of sense of smell in workers exposed for hours to decomposing cadaver or to cesspools (Amoore, 1986). Six of eight patients that were accidentally exposed to H_2S during work 2-3 years prior were evaluated for chemosensation (smell and taste) function and were found to have deficits of varying degrees (Hirsch and Zavala, 1999). The tests included an inquiry into their subjective experiences, bilateral odour detection tests with various odorants and the Chicago smell test. Most of the subjects also underwent the University of Pennsylvania Smell Identification Test, a series of 40 scratch and sniff force choice odour identification questions.

In a subchronic study of rats exposed to H_2S (0, 10, 30, or 80 ppm) for 6 hr/day, 7 days/wk for 10 weeks, animals exposed to 30 ppm had mild (25 - 50% reduction in the normal thickness of the olfactory neuron layer) to moderate (50 - 75% reduction) olfactory neuron loss and basal cell hyperplasia in the lining of the dorsal medial meatus and the dorsal and medial regions of the ethmoid recess (Brenneman et al., 2000).

A symptom prevalence survey among residents in three neighborhoods that were various distances from California petroleum refineries was conducted by Goldsmith (cited in Shusterman, 1992). In the three zones, the intensity of odour exposure was validated with olfactometry. The frequency of odour perception and degree of odour annoyance was related to the exposure zone. Significantly higher rates of self-reported eye or nose irritation, dizziness and nausea were reported by those who were bothered 'very much' or 'moderately' by the odours. Schiffman et al. (1995) evaluated 'profile of mood states', using two measures, for 44 residents exposed to a mixture of compounds included H_2S, mercaptans, aldehydes and volatile organic acids, near a large scale swine operation, and compared them to a control population. Levels of emissions were not measured. The experimental group reported significantly more tension, depression, fatigue, anger, confusion and less vigor than the control group.

Bowler et al. (1994) studied the psychological and psychophysiological sequelae in a community 3-4 months after a railroad spill of 19,000 gallons of the pesticide metam sodium in the Upper Sacramento River in northern California. When metam sodium combines with water, it breaks down to methylisothiocyanate and hydrogen sulfide, however no monitoring was done until several days after the spill. The symptoms of 705 people that reported heath problems at medical centers during the first month post-spill included headache (64%), nausea (46%), eye irritation/blurring (40%), dizziness (30%), shortness of breath (27%) and diarrhea (25%). Complaints of depression, disorientation, drowsiness, dry mouth, earache, fatigue, fever, hot flashes, irritability, memory reduction, nose bleed, numbness, pain in the arms or legs, tinnitus (ringing in the ear) and sweating were also reported at medical centers. Bowler et al. (1994) surveyed 350 spill residents and 114 non-exposed controls from a similar but unexposed town. Psychological measures used were the MMPI-2, Profile of Mood States (POMS), Impact of Event Scale (IES), Environmental Worry Scale, Perceived Social Support and Perceived Control Scales. Physiological measures were blood pressure, pulse and cortisol level. Sixty-one percent of the spill residents showed clinical abnormalities on a least one or more of the MMPI-2 clinical scales. The spill residents had significantly more worry and lower perceived social support than their matched controls. Significantly higher blood pressure and less fluctuation of salivary cortisol levels were also found. More neurological, memory and concentration, anxiety, depression, sleep disorders, headaches, visual, olfactory, dermatological, gastro-intestinal and cardiac symptoms were reported than the controls. Comparing those who smelled the substance with those who did not, increased impact of the event, greater levels of intrusion and avoidance, more affective disturbance and increased diastolic blood pressure was found. In a subgroup of matched pairs (age, education, gender, race and number of children), greater levels of depression, anxiety, and somatic symptoms in the spill residents, greater environmental worry and lower perceived social support were found.

Deane and Sanders (1977) administered health questionnaires to residents from areas with different exposures to odours from refineries and petrochemical plants. The methyl mercaptan concentration was between 50 and 655 ppb. Among those who were previously bothered by the odour, exposure to odours was associated with an increase in percent reporting dizziness, nausea, or frequent vomiting. Self-reported headache and odour annoyance was associated with residential proximity to the pulp mills, however this association did not hold for other respiratory, gastrointestinal or neurological symptoms.

In a study designed to evaluate the unpleasantness of odorants, singly and in combination, Laing et al. (1994) exposed five volunteers who had prior familiarity with odour testing procedures to H_2S (sewage/rotten eggs), isovaleric acid (rancid, sweaty, sour), butanethiol (rotting cabbage), and skatole (feces). An olfactometer was used to deliver the gases in one, two, three, or four-component mixtures. The responses were recorded on a scale (with 75 invisible divisions) indicating 'no odour' and 'extremely strong' at each end. On a scale of 75, air was rated as 8.2 - 10.4 compared to a rating of 19 for H_2S (18.7 ppb). As the H_2S concentration was increased to 43.2, 91.2 and 170 ppb, the perceived intensity worsened as indicated by the ratings of 24.3, 35.2, and 51.2, respectively. A similar pattern of responses was observed for butanethiol and skatole. However the response was 'flat' for isovaleric acid. For a 65.5 ppb exposure to isovaleric acid, a rating of 38.5 was reported. As the concentration increased (111, 231, 336 ppb), the ratings were 38.5, 44.5, and 54.9, respectively. These researchers also found that when the gases were presented as a mixture, the ratings were almost always more unpleasant than the constituents. Yet, the ability to identify individual components of a mixture declined as the number of constituents increased.

These results are comparable to those reported by Winneke (1992). Using a nine-point scale, 22 subjects rated the pleasantness/unpleasantness of exposure to H_2S. At 4.8 ppb H_2S, the subjects rated the exposure as 6.5 (+/- 0.5) out of 9.0, which represented 'very unpleasant'. At 12.6 ppb the rating worsened to 7.5/9.0; and at 44.7 ppb, the subjects rated the unpleasantness at slightly greater than 8.0 out of a 9-point scale. Similar slopes of suprathreshold intensity, which refers to the perceived strength of the odorant/irritant sensation, were found for formaldehyde and H_2S (Winneke, 1992). As the concentration of gas increased from 1.65 ppb to 52.8 ppb, the responses on a 6-point scale changed from 0.5 (midway between not perceptible and very weak) to 4.5 (midway between strong and very strong). Winneke (1992) also studied the phenomenon of adaptation (strength of sensation decreases with prolonged odorant/irritant exposure), demonstrating a well-defined adaptation between sessions of H_2S exposure. Winneke (1992) also found that H_2S (concentration range of 4 ppb to 325 ppb) produced peripheral vasoconstriction as well as pupil dilation.

Smith et al. (1999) summarized some of the literature on the effects of odour on the cardiovascular system. In general, they found that odours could induce changes in the cardiovascular system. Two broad categories of changes were described - reflex or neuroendocrine. Neuroendocrine changes included observations such as increases in catecholamine and cortisol levels that can lead to increased heart rate, blood pressure, vasoconstriction, platelet aggregation, and alterations in blood lipids. Reflex or 'aversive response' changes included apnea (cessation of breathing), cardiac slowing and a small rise in blood pressure.

Earlier work on odours identified similar associations. At the Fourth Symposium on Environmental Health Measure of Annoyance Due to Exposure to Environmental Factors (Anonymous, 1973), under the heading of 'Clinical Aspects', comments were made as follows: "*Annoyance may affect the cardiovascular system by producing clinical symptoms resulting from an increase in cardiac output, a rise in blood pressure and pulse rate and an increased myocardial oxygen consumption. The suspicion that annoyance plays a part in the causation of essential hypertension has some support also from clinical evidence. The alimentary system may react briskly to feelings of annoyance. Vomiting may be induced by stimuli which disgust; alterations of motility in the gastrointestinal tract in response to stress may produce dysphasia, flatulence, anorexia, nausea, diarrhea, colic or constipation.*" Overcash et al. (1983) noted that odors may cause nausea, vomiting, headache, shallow breathing, coughing, sleep disorders, upset stomach, appetite suppression, irritated eyes, nose and throat and mood disturbances including agitation, annoyance and depression.

A study of 2300 Dusseldorf citizens living in areas of either predominant traffic noise or industrial odour were surveyed to identify determinants of increased sensitivity to odorant/irritant compounds (Winneke, 1992). A group of 150 nonsmoking subjects pre-selected from this sample were further selected on the basis of either high or low degrees of environmental annoyance induced by traffic or odour. They were exposed to one hour of environmental tobacco smoke containing 5 or 10-ppm carbon monoxide, or noise (65 or 75 dBA [decibels A-weighting]) or odour (50 or 150 ppb H_2S) and were surveyed 3 times during the exposure period. A pronounced graded dose-response occurred for the three exposure conditions with ETS, noise and odour. All were significantly different from controls, although odour was less so. Personality traits such as anxiety, emotional liability, or extroversion/introversion were found not to be predictive. Instead, age, perceived health status, and gender were predictive factors in modulating the degree of annoyance. If subjects were employed at the source of emissions, they perceived the odour annoyance less than controls, although the difference just approached statistical significance. Also, odour exposure in terms of the frequency of odour events per year was the most important single factor to modulate odour annoyance.

2.11.6 Odour Sensitivities and Community Responses

Approximately 30% of respondents in a study of the US Environmental Protection Agency workers described unusual sensitivity to environmental chemicals (US EPA, 1989). Intolerance to low levels of environmental chemicals was also found in 19% of 114 California patients who were diagnosed by physicians as having occupational disease attributable to organophosphates (Tabershaw, 1966), in 13% of a sample of 160 consecutively admitted solvent-exposed workers (Gyntelberg et al, 1986), in 95% of 60 asthmatics (Shim and Williams, 1986), and in 80% or 105 consecutively admitted allergy clinic patients (cited in Ziem and Davidoff, 1992). Riskowski (1991) described an odour phenomenon in mixed gas environments where ammonia and H_2S odours are detectable at much lower levels than previously published odour threshold concentrations, suggesting an enhanced odour detection interaction.

2.11.7 Illnesses Attributed to Psychogenic Origins

Several reports have shown how communities or groups of people are affected by exposure to chemicals with offensive odours (Landrigan and Miller, 1983; House and Holness, 1997; Jones et al., 2000). All three cases were attributed to 'psychogenic' causes. In the first case, an odour escaping from a school latrine in the Israeli West Bank, triggered increased and wide-spread reports of headache, dizziness, blurred vision, abdominal pain, myalgia and fainting. Low levels of H_2S, mercaptans and volatile organic compounds were found, although none in concentrations adequate to account for the symptoms reported.

In the second case, sardine packing factory employees had complaints of eye and throat irritation, chest tightness, headache, and weakness, which later progressed to episodes of poor coordination and dizziness. Unusual episodes of laughing followed by crying were also reported. On several occasions, the symptoms were so severe and prevalent that the work was stopped and the operation shut down. The symptoms eventually spread to other parts of factory. Although the industrial hygiene investigations could find no cause for the symptoms, ventilation in the packing area was found to be inadequate and low levels of exposure to some odorants and irritants were measured (carbon monoxide, sulfur dioxide). All of the exposures (details were not provided) were found to be below the permissible exposure limits.

In the third case, a teacher noted a 'gasoline-like' smell, and thereafter experienced symptoms of headache, dizziness, nausea, drowsiness, chest tightness, and difficulty breathing (Jones et al., 2000). Similar symptoms were reported by several students in her room and while the classroom was being evacuated, more reported symptoms. Of 186 affected, the predominant symptoms were headache (89%), dizziness (70%), nausea (65%), drowsiness (57%), chest tightness (49%) and breathing difficulty (49%). Various emergency response and governmental agencies responded to investigate. Several days of testing of the air did not reveal any contaminants. As such, Jones et al. (2000) attributed the outbreak to mass psychogenic illness. However, as subsequent letters to the editor suggested, several alternative explanations are possible. It is also very likely that the timing of sample collection and the inadequate sensitivity of the air monitoring instruments used, limited the investigators' ability to identify possible agents.

In Michigan, in response to odor complaints from citizens, hourly reduced gaseous sulfur (TRS) data was collected downwind of a petroleum refinery complex over 700 days from 1991 to 1994 (Michigan Department of Environmental Quality [MDEQ], 1998). The TRS was believed to be an upper limit estimate of H_2S as H_2S is generally the most abundant component of TRS. Most of the hourly values were at or below the minimum analytical instrument detection level of 1 ppb and the arithmetic mean for the 4 years was about 1 ppb. Hourly concentrations above 10 ppb occurred on only 5% of the days and accounted for less than 0.4% of the hourly readings. The maximum observed hourly concentration was 44 ppb. Of 469 complaints reported to the MDEQ, 75 complaints listed associated health effects, none of which was followed up with a medical evaluation. These included 23 reports of nausea, 32 of headaches, and 9 respiratory problems. Also reported were five cases of eye irritation, one of skin irritation, one of insomnia, two of taste, and 11 miscellaneous complaints. Only seven of the 75 reports that listed health effects also had documented H_2S ambient air testing. The H_2S concentration in these 7 cases ranged from 0 ppm - 10 ppm (Michigan Environmental Science Board Hydrogen Sulfide Investigation Panel, 2000).

2.11.8 Gaps and Limitations

The cases of 'psychogenic illness' clearly demonstrate deficiencies in current monitoring practices and emphasize the need to study combined effects of exposure to multiple low level chemicals. As odours, especially aversive odours, have multiple impacts on humans, producing changes in emotion, physiology, behavior, and other responses, greater attention should be given to studying these interactions.

As has been recognized in the occupational health field for some time now, that the perception of a hazard is an important component of the hazard itself (Baker, 2000), it should be recognized that the emotional component of an odour response in ambient air quality management is equally important. The frequency of odour incidents appears to affect both the symptoms reported and also the emotional response. This relationship should be investigated further. This also suggests that the guideline should be modified to incorporate limitations on the frequency of odour incidents.

2.12 RENAL EFFECTS

Only one study was located in the past decade that specifically addressed the relationship between H_2S and renal function. Older literature was therefore sought for inclusion (Table A22; Appendix I).

Renal symptoms were reported 2.5 times more frequent in communities exposed to low levels of H_2S as compared to reference communities (Legator et al., 2001). The symptoms included on the questionnaire for the renal category included: kidney condition, bladder disease, frequent painful urination, blood in urine, dialysis of Pharisee, bladder infection, kidney infection, or other problems.

A common reported finding in the older literature appeared to be the presence of proteinuria following acute exposure (Audeau et al., 1985; Peters, 1981; Dreisback, 1980; Burnett et al., 1977; See Table A22, Appendix I).

2.12.1 Gaps and Limitations

No exposure information is available in these reports to assess what concentration produces these effects. Proteinuria and hematuria, believed to be a feature of H_2S poisoning, and proteinuria found in the case reports, suggests that glomeruli filtration has been affected. These findings may be explained by the studies of Ng and Tonzetich (1984) demonstrating an increase in mucosal permeability following nanomolar methylmercaptan exposure. In the kidney glomeruli, two basal laminae, which are only 20 - 100 nm thick, separate the blood from the urinary space. This basement membrane is thought to be the principal component of the selective permeability barrier, which enables plasma filtration as the first step in the urine formation process. As transient proteinuria is not considered to be clinically significant, additional research should be undertaken, although given a low priority, to clarify these relationships.

Table 22 Renal Effects of H_2S Exposure

Study Design / Reference	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (OR) (OR 12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.4[illegible]; 1.44 - 4.42).

2.13 REPRODUCTIVE EFFECTS

Many anecdotal reports draw attention to concerns about an association between sour gas exposures (i.e. H_2S) and altered reproductive outcomes (CASA Animal Health Workshop, 1999; Ludwig, 1997; Church, 1992; Round, 1992). However, few studies were undertaken in the decade that examined the relationship between reproduction and H_2S exposure. Associations of adverse reproductive outcomes are suggested in several studies (Waldner, 1999; Xu et al., 1998; Fateeva, 1998; Waldner et al, 1998; Scott, 1998; Stair et al., 1996; Tabacova & Vukov, 1991; Hayden et al., 1990b) and in older literature (Hemminki & Niemi, 1982; See Table A23; Appendix I) but not in others (Dorman et al., 2000).

In a retrospective study of 2853 women working at a large highly automated petrochemical plant consisting of 17 facilities reported to have low routine chemical exposure, increased risk for spontaneous abortion was associated with exposure to most chemicals (overall odds ratio [OR] 2.7; 95% CI 1.8 -3.9). Of the total group of females, there were 106 exposed only to H_2S, whose risk for spontaneous abortion was 2.3 (95% CI 1.2 - 4.4) (Xu et al., 1998). No H_2S concentrations were reported. These findings are consistent with those obtained by researchers in the former Soviet Union (Delov et al., 1997; Gainullina & Karimova, 1995) and in earlier human and animal studies (Hofmann, 1928; Vasil'eva (1973) [cited in NRC, 1981]; See Table A23; Appendix I).

Following exposure of rats to 10, 30 or 80 ppm for 6 h/day; 7 days/wk for 2 weeks prior to breeding, for a 2-week mating period, and then from gestation day 0 (evidence of copulation) through day 19, Dorman et al. (2000) found no deaths and no adverse physical signs during the study, nor any statistically significant effects on reproductive performance. Exposure did not affect pup growth, development, or performance on any of the behavioral tests. However, several findings are suggestive of significant biologic effects: an apparent dose-dependent increase in abnormal sperm, reductions in the number of live births in exposed animals, and increased post-implantation losses in exposed animals compared to controls. Yet researchers in the former Soviet Union have found reproductive effects of polysulfide of natural gas and condensate on rats at H_2S concentrations in the range of 1.7 +/- 0.09 ppm (Fateeva, 1998) suggesting that chemical mixtures containing H_2S may be more toxic than H_2S alone. These findings are consistent with earlier studies of H_2S and CS_2-exposed rabbits (Wakatsuki & Higashikawa, 1959; Table A23; Appendix I).

In gravid rat dams exposed to H_2S (20, 50 and 75 ppm) from day 6 of gestation until day 21 postpartum, a dose-dependent delay in delivery time was observed in all exposed dams (Hayden et al., 1990b). A 6.8% increase was observed with 20-ppm exposure, 20% increase with 50 ppm, and a maximum increase of 42% at 75 ppm. Incidentally, Beruashvilli (1980); [cited in Beauchamp et al., 1984] reported that rats administered 'thermal' water from the Georgia province of the former Soviet Union 'tends to depress the spermatogenic index'.

Several anecdotes were described by Nikiforuk (2001). The first was of an incident in which the Ludwig's attribute reproductive problems to a release of 59 m^3 of raw sour gas into the air at the Ranchmen's site due to a low-pressure separator gauge glass burst. An H_2S monitor measured 8 ppm 200 meters east of the site. The Ludwig's reported the odors to the provincial agency. That spring, two observations prompted concern about the reproductive effects of sour gas. Harmony Ludwig noticed reproductive problems in her flock of sheep. In the previous 5 years, she had only seen 2 abortions whereas this spring, many of the sheep aborted and more than 20 of 55 lambs born were stillbirths. Similar problems were occurring with the goats, which also grazed on the field next to the well. Also that spring, Mamie Ludwig had a miscarriage in her first trimester, after 11 previously healthy pregnancies.

The second was of the connection made between the 787,000 m^3 gas released from 8 flares during the first 3 months of Kara's pregnancy, and her headaches, light-headedness and nausea before she lost her baby. One goat and one sheep spontaneously aborted at the same time (Nikiforuk, 2001; p 43).

In the first couple of weeks of operation of a Norcen sour gas plant 6 kilometers away, Bill and John Bocock, lost 8 cows, several other animals produced no milk or became hyperactive or dumb (Nikiforuk, 2001; p 97). That same year, several months after a period of sour gas well flaring, the Bocock's started to see abnormal sexual behavior in cows, bulls, heifers, tomcats, wild ducks and crows. Their herd's twinning rate increased from the usual rate of 1% to 6%. In subsequent years, reproductive problems persisted. Only 11 of 45 calves born in 1996 were healthy at birth. Abnormal sexual behavior including decreased bonding of cows with newborn calves and increased twinning was also reported after a sour gas/condensate leak by Stair et al. (1996).

2.13.1 Gaps and Limitations

There is considerable discrepancy between the reproductive effects reported in animal experiments and those given in the anecdotal reports. Some key differences between the laboratory experiments and the environmental exposures are the exposure condition dynamics and the chemical mixture. In the former, a stable concentration of H_2S only is delivered to the animal for a constant time period whereas in the real-life scenarios, exposures, due to the dispersive nature of the daytime atmosphere, are characterized by widely fluctuating concentrations of a mixture of gases. The exposure dynamics may be relevant as many reproductive hormones have cyclical oscillatory patterns in the bloodstream and disturbances of those oscillations are known to affect normal functions (Lloyd and Rossi, 1993).

Further research should be conducted *in vivo* and *in vitro* using a variety of systems and on other animals in addition to rats, to clarify the reproductive effects of H_2S alone and in combination with other gases and vapors. These should include: raw natural gas, carbon disulfide, carbon monoxide, mercaptans, aromatic and aliphatic hydrocarbons.

Effects of reduced sulfur compounds on twinning should also be addressed. Early *in vitro* research suggests that exposure of eggs to reduced sulphur agents affects twinning rates (Mazia, 1959). Exposure of sea urchin eggs to mercaptoethanol, only after the eggs passed metaphase, gave rise to twin embryos and only eggs that were exposed during their actual cleavage produced twins.

2.14 RESPIRATORY EFFECTS

Many studies located during the past decade have provided a better understanding of respiratory health effects, especially the effects of long-term low level H_2S exposure (Table 24). Many respiratory effects have been reported following acute exposures. These include: increased respiratory symptoms (Parra et al., 1991; Haahtela et al., 1992; Bomans et al., 1997; Hessel et al., 1997; Salano and Copello, 1998; Hessel & Melenka, 1999), obstructive pattern from lung function tests (Van Aalst et al., 2000) residual lung volume reductions (Buick et al., 2000; Parra et al, 1991), pulmonary edema (Trubnikov et al., 1996;Tanaka et al., 1999; Chaturvedi et al., 2000), injured bronchi (Parra et al., 1991;Trubnikov et al., 1996;Tanaka, 1998; Van Aalst et al, 2000; Hessel & Melenka,1999), bronchial hyperreactivity (Bomans et al.,1997) and reactive airways syndrome (Cormier et al., 1996 ; Bomans et al., 1997).

Anecdotal reports of severe respiratory reactions requiring medical attention in children corresponding to the timing of nearby flaring have also been made (Jenson, 1999). Similar reports are also found in earlier literature (See Table A24; Appendix I).

Following chronic exposures, increased hospitalizations for asthma symptoms (Kathman et al., 2001; Rossi et al., 1993), increased respiratory symptoms (Wingren et al., 1991; Marttila et al, 1994b; Anonymous, 1995; Hessel et al., 1997; Legator et al., 2001; Mostachni et al., 2000), bronchial obstructions (Jappinen et al., 1990; Hessel & Melenka, 1999) and reactive airways disease (Hessel et al., 1997; Marttila et al., 1995) have been reported. Sewer workers (Richardson, 1995) and pulp and paper workers (Salano & Copello, 1998) appear to be at greater risk for developing chronic lung disease. Similar reports are also found in earlier literature (See Table A24, Appendix I).

Table 23 Reproductive Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Dorman et al (2000) Rats were exposed to H_2S at various concentrations (0, 10, 30, 80 ppm; 6 hr/day; 7 days/wk) for 2 wks prior to breeding, for a 2-wk mating period, and then from gestation day 0 (evidence of copulation) through day 19. Exposure of dams and their pups resumed between postnatal day 5 and 18. Adult male rats were exposed for 70 consecutive days. The animals were subjected to various neurological tests (motor activity, passive avoidance, functional observational battery, acoustic startle, neuropathology), at various times during the exposure period. At the end of exposure period, the animals were sacrificed and their tissues examined.	There were no deaths and no adverse physical signs during the study, nor any statistically significant effects on reproductive performance. Exposure to H_2S, reportedly did not affect pup growth, development, or performance on any of the behavioral tests. However, the percentage of abnormal sperm increased corresponding to increased exposure. The number of live births decreased, although not statistically significant, corresponding with increased exposure. The number of post-implantation losses was greater, although not statistically significant, in the exposed groups as compared to the non-exposed group.
Waldner (1999) Four study designs were used to evaluate the impacts of sour gas emissions on cattle health.	Increased twinning, calving intervals, abortions and stillbirths were associated with emissions from the Caroline gas plant. Increased calving intervals and stillbirths were associated with active gas wells and large facilities flaring activities.
Fateeva et al. (1998) Experiments were conducted on 80 non-inbred female rats. Animals were exposed to polysulfide of natural gas and condensate daily for 4 hrs, 5 times a week for 20 days. The mean concentration of chemical components was 1.7+/-0.09 ppm as calculated for H_2S.	The findings indicate that this substance has an adverse effect on reproductive function (details not available).
Xu et al. (1998) A retrospective study of 2853 women employed at a large petrochemical complex was done to assess the association between petrochemical exposure and spontaneous abortion.	Significantly increased risk of spontaneous abortion for working in all of the production plants with frequent exposure to petrochemicals (OR 2.7; 95%CI 1.8 - 2.9). In the specific chemical exposure analyses, an increased risk of spontaneous abortion was found with exposure to most chemicals, and the results for benzene (OR 2.5; 95%CI 1.7 - 3.7), gasoline (OR 1.8; 95%CI 1.1 - 2.9) and hydrogen sulphide (OR 2.3; 95%CI 1.2 - 4.4) were significant. These results are especially important since the authors note that the petrochemical plant in the study has reached a stage of high automation and routine chemical exposure is maintained at a very low level.

Scott (1998) Geographic information systems technologies were used to investigate the effects of emissions from sour gas processing plants and oilfield batteries on the health and productivity of beef cow-calf and dairy herds. Dispersion modeled emissions were used as exposure variables.	A statistically significant small negative effect of peak exposures to sour gas emissions on the age at first calving was found. A tendency toward increased calving interval was also associated with increased emissions. Evidence of a negative association between the sour gas plant emissions and the level of twinning and calving season profile was found. No significant associations between solution gas flaring volumes and dairy health or productivity was found. No significant associations were found between sour gas plant emissions and culling, stillbirth, calf mortality or calf-crop weaned.
Waldner et al. (1998) A prospective cohort study was undertaken to measure associations between a leak of sour natural gas (> 30% H_2S) from a pipeline in a river valley and the health of beef cattle in the intensively ranched surrounding area.	No associations were found between total herd calf mortality and herd distance from the leak, wind-aided exposure, location in the river valley, signs of irritation consistent with gas exposure or reports of odors of gas at the time of leak. However, 2 of the 18 study herds had a combined total calf mortality (abortions, stillbirths, and postnatal deaths) of 12.5% representing 48% of the total herd mortality in the study herds. The incidence of twin births was highest in the 2 herds reporting increased calf mortality. In the herd with the highest relative calf mortality, being a twin significantly increased the likelihood of death for all ages, for death during the first 24 hours after birth, and for calves classified as weak at birth.
Delov et al. (1997) An analysis of the outcomes and course of pregnancy and delivery in 102 women working in a sulfurated hydrogen gas processing industry during 5 years was done.	Gas with high sulfurated hydrogen content affects pregnant women, newborns and children during first year of life. There was a great number of premature labors, feto-placental insufficiency, asphyxia and hypotrophy of newborns. A correlation between pathological conditions of the pregnant woman and newborn health was observed.
Stair et al. (1996) The health of 460 beef cattle and their calves within 3 miles was assessed after a pipeline leak of volatile components of crude sour petroleum, emissions from burning sour condensate and steam washing of gravel occurred at the Red Deer River.	Clinical observations included irritation of mucous membranes, abnormal sexual behavior, decreased bonding of cows with newborn calves and failure of calves to thrive. Some cows had proprioceptive-locomotor deficits attributed to the central nervous system and an increased rate of twinning was present.
Gainullina & Karimova (1995) Women working at oil-processing enterprises are exposed to toxic chemicals.	The women demonstrate functional disorders of nervous, cardiovascular systems, and disturbances in menstrual and childbearing functions.

Tabacova & Vukov (1991) In areas contaminated by metallurgy, chemical and petrochemical industrial sources, rates of spontaneous abortion, malformation, stillbirths, prematurity, and early neonatal morbidity and mortality were determined for a 5-yr retrospective period which included 238,221 births and 30,579 spontaneous abortions. Exposures via air (SO_2, NO_2, H_2S, particulates, Pb aerosols), soil and water (e.g., Pb, Cd, Cr, Mn, Zn) were studied in parallel.	Significant correlations were found between the rate of malformations at birth and Cd, Cr and particulate pollution as well as Zn deficiency. Also, Zn was negatively correlated with the rate of spontaneous abortion. Strong positive correlations were found between Cd and stillbirths, and Cr and premature births. Pb exposure was not correlated with the above outcomes, but was related to maternal toxemia and neonatal morbidity. Ambient H_2S also was related to maternal toxemia, and Mn exposure contributed to increased prematurity and neonatal morbidity. Ambient NO_2 pollution was related to prematurity and neonatal mortality. The natural radiation background did not influence any of the reproductive parameters studied.
Hayden et al. (1990b) Gravid rat dams were exposed to H_2S (20, 50 and 75 ppm) from day 6 of gestation until day 21 postpartum. Weights were measured at gestation days 1, 5, 9, 13, 17, 21, and post-partum days 1, 7, 14, and 21.	Maternal body weight gains (58% increase) were similar for both the control and exposed dams. However, a trend toward a dose-dependent reduced weight gain, although not statistically significant, is apparent. Food intake was reduced during the first 4 days of exposure to 50 ppm H_2S and during the first 8 days of exposure to 75 ppm H_2S, however recovered after several days. Brain and liver weights did not differ between controls and the various treatment groups. Length of gestation, viability, and litter size or male to female ratio of newborn pups were not altered. Nor was the time to incisor eruption, eyelid opening or surface righting altered. However, animals exposed to 20 ppm H_2S had hastened pinna detachment and animals exposed to both 20 and 50 ppm had hastened hair development, the lower concentration giving the shorter period. No differences were found between exposed and control pup liver, brain or total body weights. No differences were found between exposed and control rat pups or dams for total liver protein or DNA accumulation. A dose-dependent delay in delivery time was observed in all exposed dams. A 6.8% increase was observed with 20-ppm exposure, 20% increase with 50 ppm, and a maximum increase of 42% at 75 ppm.

Long-term low-level H_2S exposure in a geothermal area was associated with increase in 'other diseases of the upper respiratory tract' (SIR 1.27; 95%CI 1.20 - 1.33) (Bates et al., 1998). Six air monitor workers had throat irritation after a 5-hour exposure to 90 ppb H_2S downwind of an oil refinery (TNRCC, 1998). Increased respiratory symptoms were found for the respiratory system (OR 11.92; 95%CI 6.03-25.72) in residents living downwind of a geothermal plant in Hawaii and in the vicinity of industrial wastewater ponds in Texas (Legator et al., 2001). Compared to the reference communities, significantly increased respiratory symptoms were reported which included wheezing, shortness of breath, persistent cough, and bronchitis. Shortness of breath was reported in about 40% of the exposed populations followed by persistent cough in 35% and wheeze in about 27%. H_2S concentrations in the geothermal area ranged from low ppb with peaks of 200 - 500 ppb whereas the concentrations measured 1 mile from the ponds were 335-503 ppb for a maximum 8-hr measurement, 101-201 ppb for a maximum 24 hr measurement, and 7-27 ppb for an annual average.

Wing and Wolf (2000) also found higher upper respiratory tract symptoms in communities exposed to intensive livestock operations. Compared to the reference community, higher rates of stuffy nose/sinuses (2.97 fold), runny nose (5.18 fold), burning nose/sinuses (1.99), sore throat (3.64), scratchy throat (2.09), mucus/phlegm (3.91 times), and excessive coughing (4.74 times) were found for people living near hog operations. Residents living near cattle operations reported episodes of excessive coughing 2.15 times more than the reference community. No H_2S concentration measurements were provided. Increased respiratory symptoms were also found among Iowa residents living within 2 miles of a 4000-head swine operation (Thu et al., 1997).

In pulp mill communities where the annual mean TRS ranged from 2- 14 ug/m3, increased risks, which were dose-dependent for cough, nasal and pharyngeal symptoms and respiratory infection were found (Partti-Pellinen et al., 1996; Marttila et al., 1995; Jaakkola et al., 1991; Jaakkola et al., 1990). Risks for acute respiratory infections, and nasal symptoms and cough decreased significantly following implementation of malodorous emissions control measures which reduced the TRS from 11 ug/m3 to 6 ug/m3 (Jaakkola et al., 1999). Compared to the non-polluted community, increased symptoms of cough nasal symptoms were found in children exposed during the previous 4 or 12 weeks to polluted air (Marttila et al., 1994b).

In a time-series study of children and adults exposed to emissions from industrial sources, liquid-waste treatment lagoons, and municipal sewer lift stations in Dakota City and South Sioux City, a positive association was found between asthma hospital visits and 1-day lagged TRS levels for children under 18 years of age (Kathman et al., 2001). A positive association was also found between asthma, hospital visits for all respiratory diseases and 1-day lagged H_2S levels and 1-day lagged TRS. No association was found for adults. Previous environmental sampling in this community had measured H_2S up to 1.37 ppm (15 min sampling period) and TRS up to 2.22 ppm (30-minute sampling period).

The ATSDR (1999b) investigated complaints associated with a large landfill on Staten Island. They measured the air quality and solicited health dairies from residents of the area adjacent to the landfill. Analysis was done to determine if there was a correlation between measures of landfill emissions and respiratory health of participants. The 15-min maximum for H_2S per day, ranged from non-detected (< 2 ppb) to 33 ppb, averaging 6.6 ppb. The results did not indicate a relationship between measures of H_2S and respiratory morbidity during the study period. The results, however, indicated a significant association between the perception of odor of rotten eggs or garbage (as documented in the daily diaries) and measures of respiratory morbidity (respiratory symptoms, and change in peak-flow and medication use).

The health effects of malodorous sulfur compounds in two polluted and one 'non-polluted' community's were assessed in a prospective cohort study (Jaakkola et al., 1999). The pulp mill in the polluted town had planned to install pollution reduction equipment to reduce emissions by 50%. This provided the researchers with the unique opportunity to conduct a prospective cohort study. In the severely polluted community, the annual ambient air concentration of total reduced sulfur compounds decreased from 11 to 6 ug/m^3. Compared with the non-polluted community, the relative decrease in acute respiratory infections, adjusted for a change in smoking habits, was 0.53 episodes/person-year (95%CI 0.22- 0.83) in the severely polluted community, and 0.36 episodes/person-year (95%CI 0.06 - 0.66) in the moderately polluted community. The frequency of nasal symptoms and cough also decreased significantly.

2.14.1 Gaps and Limitations

Few major gaps or limitations are identified in the Scandinavian studies. The study designs were diverse and included cross-sectional, retrospective, prospective cohort, and time-series analysis. Although exposures were usually obtained by area, as opposed to personal monitors, several communities with range of pollutant concentrations were studied, reducing the likelihood for misclassification bias. In most studies, adjustments were made for confounders. Collectively, the Scandinavian researchers have consistently shown dose-dependent respiratory effects at very low ambient levels of H_2S (1-5 ppb) and TRS (1-5 ug/m^3). As such, the consistent findings of adverse respiratory health effects at lower concentrations than previously recorded are believed to be real:

- After controlling for potential confounders, exposure to 6 ppb H_2S and 1 - 2.5 ppb methyl mercaptan (as annual means) was associated with dose-dependent increases in eye, nasal symptoms and cough (Jaakkola et al., 1990).
- After controlling for potential confounders, exposure to 1.6 ppb H_2S (annual mean concentration) in a mixture of other pollutants (SO_2 13 - 23 ug/m^3; NO_x 15 ug/m3 and particulates 31 ug/m^3), both young and older children had more upper respiratory tract infections during the previous 12 months than the reference cities (Jaakkola et al., 1991).
- Significantly increased eye, cough or pharyngeal symptoms and difficulty breathing was found during the release period in residents living downwind of a paper mill that released H_2S for 2 days (24-hour average H_2S concentrations were 35 and 43 ug/m3 (25 and 31 ppb) and a 4-hour maximum was 135 ug/m^3 (96 ppb)), as compared to the reference period (Haahtela et al., 1992).
- Significantly increased hospital visits for asthma within the same week were found to correlate with NO_2 however those for SO_2, TSP and H_2S were also significant. Over the year, the H_2S daily mean was 3.1 ug/m3 (2.2 ppb) and the range was 0-34 ug/m^3 (0 - 24 ppb) (Rossi et al., 1993).

- Increased symptoms of cough, nasal symptoms, in children were found in moderately and severely polluted cities with annual mean H_2S levels of 1-8 ug/m3 (0.7 - 5.7 ppb) and 2-month mean of 2 - 4 ug/m^3 (1.4 - 2.9 ppb) occurring with annual mean methyl mercaptan levels of < 1 - 5 ug/m3 (0.5 - 2.5 ppb) and annual mean SO_2 concentrations of 1 - 2 ug/m^3 (0.4 - 0.8 ppb) (Marttila et al., 1994b).
- Dose-dependent increases in nasal (stuffy or runny nose) and pharyngeal irritation was found in a population living near a pulp mill emitting low daily levels of malodorous sulfur compounds (TRS 10 - 30 ug/m^3) (Marttila et al., 1995).
- After controlling for confounders, increased risks for cough and respiratory infection in the last year were found and increased nasal symptoms were found but only for the previous 4-week period in an area where annual TRS and SO_2 concentrations were measured 2-3 ug/m^3 and 1 ug/m^3 (0.4 ppb) respectively (Partti-Pellinen et al., 1996). The TRS concentrations equate to ~1.2 ppb H_2S as an annual mean, assuming that 2/3 of the TRS is H_2S.
- After adjusting for change in smoking habits, reductions in acute respiratory infections and frequency of nasal symptoms and cough decreased significantly after installation of pollution reduction equipment at a pulp mill which reduced the annual ambient air concentration of TRS from 11 to 6 ug/m^3 (Jaakkola et al, 1999).

The possibility of acquired bronchial reactivity following exposure to H_2S should be examined both in laboratory and clinical studies.

2.14.2 Edema, Inflammation and Airway Hyper-responsiveness

Lung edema is a common clinical finding in oxidant-induced organ injury (Holman and Maier, 1990), in workers exposed to H_2S (Van Aalst et al., 2000; Chaturvedi et al., 2000; Tanaka et al., 1999) to other industrial agents (Corasco et al, 1973), and in H_2S -exposed animals (Khan et al., 1995; Kohno et al., 1991; Green et al., 1991; Green et al, 1990). Yet the mechanisms for edema development are not well understood (Holman and Maier, 1990). A common finding is loss of vascular endothelial cell integrity and subsequent leakage of fluids into interstitium.

Presence of edema in the lung may be explained, in part, by simultaneous alterations of several processes such as: i) H_2S-induced tissue injury stimulating an inflammatory process; ii) H_2S-induced changes in mucosal permeability (see Tables 24 and 26); and iii) H_2S-induced reductions in ATP production (Holman and Maier, 1990).

Release of cytosolic enzymes into the extracellular fluid of the lung or into plasma is evidence of cell damage resulting in leakage (Hohnadel, 1984), and cell death (Bremner, 1984). Increased protein and enzyme activities are observed in lung fluid (Green et al., 1991; Khan et al, 1995) after high exposures (200 - 400 ppm H_2S for 4 hours) confirming findings in earlier studies (Lopez, 1987, 1988; Table A7.14.3: Appendix I). More subtle alterations were observed following a single 4-hour exposure to 10 ppm H_2S exposure (Lopez, 1987). Compared to controls, LDH activity was increased, although not statistically significant, in broncheolar lavage fluid one hour after exposure and continued to increase to 44 hours after exposure (Lopez, 1987). Cell leakage, indicated by release of LDH from lung fibroblasts grown in tissue culture, was evident at concentrations of 10 nM sodium sulfide (Hayden et al., 1990a). These results are in agreement with recent (Ratcliff and Johnson, 1999) and previous findings that nanomolar concentrations of reduced sulfur compounds increase membrane permeability (Ng and Tonzetich, 1984; See Table 9.2; Appendix I).

In the lung alveoli, a layer consisting of two basal laminae, which is only 20 - 100 nm thick, separates the blood from the air space. Type III collagen in basal laminae and fibronectin are disulfide-linked (Goldberg and Rabinovitch, 1988), and as such, are susceptible to cleavage by H_2S. Collagen and fibronectin have also been found to strongly induce interleukin-1 (IL-1) and IL-8 expression in human monocytes (Graves & Roman, 1996; Schiffer et al., 1999). IL-1, and many other cytokines are important inflammatory mediators. Cytokines are reported to induce lung edema include cysteine-leukotrienes (Cys-LT)[24], platelet activating factor (Lotvall et al., 1991), bradykinin (Rogers et al., 1990), substance P (Rogers et al., 1988), hydroxyl radical (Lei et al., 1996) and nitric oxide (Kuo et al., 1992).

Many aspects of airway function, including smooth muscle tone, epithelial cell function, mucous secretion, bronchial flow, permeability and inflammatory mediator release are under autonomic nervous system control (Barnes, 1986). C-fiber nerve endings may be exposed following epithelial damage, which are then stimulated by inflammatory mediators, such as bradykinin. This initiates a neurogenic inflammatory process that may result in a local reflex with release of sensory neuropeptides which together could account for bronchoconstriction, microvascular leakiness causing edema and plasma extravasation, mucous hypersecretion and possibly also chemotaxis and activation of inflammatory cells (Meggs, 1995).

Acid gases such as H_2S can produce direct irritant effects on mucous membranes (Leung and Paustenbach, 1988) and also appear to be associated with development of airway hyper-responsiveness (Kennedy, 1992). Jappinen et al. (1990) found 2/10 asthmatics experienced greater than 30% resistance and conductance after inhalation of 2 ppm H_2S for 30 minutes. Earlier animal studies also reported evidence of increased bronchial reactivity after repeated exposure to 1 ppm (Hulbert et al., 1988; See Table A25; Appendix I). Substance P, one of the mediators in neurogenic inflammation, is limited by a zinc metalloenzyme, neutral endopeptidase (Maggi, 1993; Rothwell & Hopkins, 1995; Meggs, 1999). As the enzyme's active site configuration includes an activated water molecule, similar to carbonic anhydrase, which is susceptible to inhibition by sulfide, substance P-mediated actions may be sustained following H_2S exposure.

Hessel's findings (1997), in addition to those of Buick et al. (2000), Boman (1997) and Hulbert et al. (1989), are consistent with the evidence that Kennedy (1992) presented that suggests airway hyper-responsiveness may be acquired as a result of exposure to irritants. Other factors, such as the variable effect, relaxation or contraction, of H_2S on smooth muscle, however are also likely involved.

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[24] Constitutive production of cysteine-containing leukotrienes may elicit increased airway edema (Henderson, 1994). Cys-LT actions are mediated via G-protein coupled receptors, present on human lung, bronchial smooth muscle and pulmonary vein tissue with binding affinities in the pM to nM range (Nicosia et al., 2000). G-protein-coupled receptors contain a pair of conserved cysteine residues, one in the first and the other in the second extracellular loop (Schulein et al., 2000), which are believed to establish the correct ligand binding domain configuration. Four extracellular cysteine residues have been shown to be critical for high affinity binding sites (Dohlmann et al., 1990) and treatment with dithiothreitol or other thiol compounds caused its functional activation in the presence or absence of agonist ligands (Pedersen and Ross, 1985).

2.14.3 Chronic Lung Disease

Possible explanations for the chronic respiratory effects associated with H_2S are considered. Hydrosulfide anion or its oxidation product sulfite anion are strong S-nucleophiles[25] (Roy and Trudinger, 1970, p 14), and may:

a) Oxidize the methionine[26] at the active site of alpha-1-anti-trypsin, an enzyme in the lung that plays a key role in balancing protease/anti-protease activities;
b) Disrupt methionine's carbon-sulphur bonds;
c) Cleave the disulfide bonds joining the alpha1-antitrypsin subunits; or
d) Modify or cleave alpha-1 protease inhibitor, an enzyme which inhibits neutrophil elastase, to produce an inactive form or cleaved into subunit derivatives[27].

The significantly elevated risks of chronic obstructive lung disease (Richardson, 1995) and the increased symptoms (Nethercott and Holness, 1988) reported in sewage workers may also be due to neutrophil activation by bacterial endotoxin producing an enhanced production of the neutral protease and elastase (Rylander and Burrell, 1988), further disrupting the protease-anti-protease balance. Reduced sulphur compounds found in sewage environments may increase mucosal permeability, allowing endotoxin to gain entry into the lung thereby facilitating an inflammatory process. The combination of effects may act synergistically to stimulate cytokine production and initiate a chronic inflammatory process.

2.14.4 Breathing Control

Almeida and Guidotti (1999) administered sodium hydrosulfide (NaHS) to anaesthetized rats by femoral intravenous, or carotid intra-arterial injections to induce apnea and found that the peripheral route of sulfide delivery is five times more effective at inducing apnea compared to direct delivery of sulfide to the brain (ED_{50} 0.6 and 3.0 mg/kg respectively). Since lidocaine applied to the vagus nerve prevented apnea, the transmission between lung and brain was considered to be essential, and the lung was identified as the primary site of action of H_2S. Bicarbonate, administered intravenously or intraperitoneally at the same time as the sulfide, appeared to prevent the apnea. These results suggest a possible peripheral receptor is involved as the primary mechanism of apnea.

A direct link between cytochrome c oxidase enzyme inhibition and apnea has not been established (Almeida and Guidotti, 1999). However, recent work by Huckstorf et al. (2000) does suggest a connection. These researchers reported that cytochrome *a* is the primary oxygen sensor for triggering chemoreceptor discharge in a response to hypoxia. They recorded light absorption spectra from hemoglobin-free rat carotid bodies while simultaneously recording the chemoreceptor discharge of the sinus nerve. The spectra were compared with various isolated cytochrome preparations (c, b558, b563, aa3, a592). They found an association between the cytochrome a592 absorption spectrum and the chemoreceptor discharge, providing support for the hypothesis that a cytochrome a species is a primary oxygen sensor protein for triggering chemoreceptor discharge in response to hypoxia.

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[25] A nucleophile is a molecule that has electrons to share.

[26] The methionine residue is known to be critical for the normal functioning of the alpha-1-anti-trypsin enzyme - that is to limit the destruction of lung tissue by neutrophilic enzymes.

[27] Tyagi (1991) found that thiol-modifed alpha-1-protease inhibitor derivatives are dissociable and reversible competitive inhibitors with Ki values in the range of 6 - 7 nM. Removal of the thiol modifications restores the rapid irreversible mode of inhibition.

Table 24 Respiratory System Effects: Human Studies

Reference / Study Design	Key Findings
Legator et al. (2001) A multi-symptom health survey was administered to two communities exposed to low levels of H_2S and to three reference communities. The exposed communities were downwind of an industrial wastewater-settling pond or in a geothermal area of Hawaii.	Compared to the reference community, 9 of 12 symptom categories had odds ratios greater than 3.0. Self-reported symptoms related to the central nervous system had the highest odds ratio (12.7; 95% CI 7.59 - 22.09). Increased symptoms were also found for the respiratory system (OR 11.92; 95%CI 6.03 - 25.72) and for the blood system (OR 8.07; 95%CI 3.64 - 21.18). Lower symptom risks were found for muscle/bone (OR 3.06; 95% CI 1.99 - 4.77); for skin (OR 3.6; 2.27 - 5.82); for immune system (5.35; 3.36 - 8.74); for cardiovascular (2.03; 1.33 - 3.12); for digestive (4.05; 2.44 - 6.96); teeth/gums (6.31; 3.46 - 12.32) and for the urinary system (2.48; 1.44 - 4.42).
Kathman et al. (2001) The association between levels of TRS and H_2S and hospital visits for asthma, all respiratory diseases, and all digestive diseases among children and adults in Dakota City and Sioux City was studied. A time series analysis for daily hospital visits and measures of TRS and H_2S were done.	A positive association was found between asthma hospital visits and 1-day lagged TRS levels for children under 18 years of age. A positive association was found between asthma, hospital visits for all respiratory diseases, and 1-day lagged H_2S levels and 1-day lagged TRS. No association was found for adults. Since 1997, H_2S levels up to 1.375 ppm (15-minute sampling period) and TRS levels up to 2.26 ppm (30-minute sampling period) have been measured in this community.
Wing and Wolf (2000) Residents of 3 rural communities (one near an intensive hog operation, one near two intensive cattle operations and a third without livestock operations that use liquid waste management systems) were surveyed to assess health symptom and quality of life indicators.	Residents living near hog operations reported episodes of stuffy nose/sinuses 2.97 times, runny nose 5.18 times, burning nose/sinuses 1.99 times, sore throat 3.64 times, scratchy throat 2.09 times, mucus/phlegm 3.91 times, and excessive coughing 4.74 times more than the reference community. Quality of life indicators (such as unable to open windows or go outside) in hog operation areas were 12 - 14 times worse than the referent community. Residents living near cattle operations reported episodes of excessive coughing 2.15 times more than the reference community.
Buick et al. (2000) Comprehensive lung function tests (spirometry, flow volume curves, static lung volumes, and CO diffusion capacity) were performed on 47 aircraft factory workers accidentally exposed to H_2S generated from accidental mixing of sodium sulfide and sulphuric acid.	Measurements were made within 4 months of H_2S exposure. 23% of the 47 workers had reductions in their residual volumes.

Van Aalst et al. (2000) Case report of two acutely exposed workers (48 and 40 years old).□	The first worker's pulmonary function tests done on day 6 in hospital showed an FVC of 3.67 (79%) and a FEV1 of 2.63(76%) which improved at the one-month follow-up to FVC 5.51 (119%); FEV1 of 3.95 (115%). The second worker's chest x-rays showed bilateral fluffy infiltrates consistent with noncardiogenic pulmonary edema. Bronchoscopy performed at 24 hours showed significant edema and diffuse petechiae and patchy mucosal erosions as far as the bronchoscope could be passed. 11 days after injury, pulmonary function tests showed and FVC of 3.78 (74%) and an FEV1 of 2.95 (76%) and remained at similar levels at a one-month follow-up FVC of 3.68 (72%); FEV1 of 2.98 (77%).
Mostachni et al. (2000) Symptom frequencies and pulmonary function tests were performed on workers employed 2- 9 years at the Kangan Sour Gas Refinery in Iran. Age, sex, and smoking status-matched controls were selected from distribution and transportation of refined oil workers.	Respiratory symptoms of persistent cough, persistent phlegm, and tightness in chest, occurred more frequently among the sour gas refinery workers as compared to controls. No differences in pulmonary function test parameters were observed between the two groups.
Chaturvedi et al. (2000) Case report of fatal exposure to H_2S. A male truck driver had accidentally transferred sodium H_2S from his tanker truck into a tank containing 4% sulfuric acid and iron sulfate.	Blood sulfide levels were measured at 1.68 ug/mL (about 34 times higher than the sulfide concentration expected in the blood of normal subjects (< 0.05 ug/mL)). Prevalence of pulmonary edema, and congestion in the lungs and kidneys was found on internal exam. Microscopically, passive congestion was evident in the lungs, spleen, kidneys and adrenal glands.
Hessel & Melenka (1999) Case report of a 50-yr old oilrig worker with a history of 2 knockdowns (1981, 1988). This worker had no history of asthma or allergic disorders and was a lifetime non-smoker.	Chest tightness and shortness of breath that were made worse by exposure to chemical fumes, cigarette smoke and other irritants was reported. The worker reported that after the 1988 episode, he has persistent headaches and has noticed a reduction in his cognitive ability, power to concentrate, and has become more quick- tempered and irritable. A full neurological examination including cranial nerves, visual fields, muscle tone, deep tendon reflexes and a sensory examination was negative. A psychological examination revealed a man with a somewhat irritable disposition. His attempt at serial sevens was accurate but extremely slow, even with prompting and his reproduction of a 3-dimensional box was limited and his copying technique was poor. Lung function tests suggested an obstructive pattern. Bronchoscopy showed inflamed and friable airway mucosa.

ATSDR (1999b) The ATSDR investigated complaints associated with a large landfill on Staten Island. They measured the air quality and solicited health dairies from residents of the area adjacent to the landfill. Analysis was done to determine if there was a correlation between measures of landfill emissions and respiratory health of participants.	The 15-min maximum for H_2S per day, ranged from non-detected (< 2 ppb) to 33 ppb, averaging 6.6 ppb. The results did not indicate a relationship between measures of H_2S and respiratory morbidity during the study period. However, there were results that indicated a significant association between the perception of odor of rotten eggs or garbage (as documented in the daily diaries) and measures of respiratory morbidity (respiratory symptoms, and change in peak-flow and medication use).
Jaakkola et al. (1999) The health effects of malodorous sulfur compounds in two polluted and one 'non-polluted' communities were assessed. The pulp mill in the polluted town had planned to install pollution reduction equipment to reduce emissions by 50%. This provided the researchers with the unique opportunity to conduct a prospective cohort study.	In the severely polluted community, the annual ambient air concentration of TRS compounds decreased from 11 ug/m^3 to 6 ug/m^3. Compared with the non-polluted community, the relative decrease in acute respiratory infections, adjusted for a change in smoking habits, was 0.53 episodes/person-year (95%CI 0.22- 0.83) in the severely polluted community and 0.36 episodes/person-year (95%CI 0.06 - 0.66) in the moderately polluted community. The frequency of nasal symptoms and cough decreased significantly.
Tanaka et al. (1999) Case report of 3 workers exposed to H_2S.	Chest x-rays showed pulmonary edema in all patients. Serial bronchoscopy suggested that inhalation of lower concentrations can produce severely injured bronchi.
Salano and Copello (1998). A cross-sectional epidemiologic study of a group of workers employed in sewer network maintenance and urban wastewater treatment plants.	The relative risk of alterations in respiratory function (both instrumental and clinical findings) was increased among the water treatment workers.
Boev et al. (1998). The physiological and biochemical states of children and adults who resided in the vicinity of the sulphide-containing gas processing plant were studied.	The chemical agents emitted by the plant were found to have adverse effects of the children's functional status, namely, decreased vital capacity of the lung, mental performance, retarded sensory-motor responses, altered enzymatic system activity.

TNRCC (1998) Six workers were exposed to a mean concentration of 0.09 ppm H_2S for approximately 5 hours in a monitoring van downwind from an oil refinery	Persistent odors, eye and throat irritation, headache and nausea were observed in the workers.
Bates et al. (1998) Cancer registry and hospital discharge data were used to compare rates of cancer and disease in Rotorua, New Zealand with the rest of New Zealand. Rotorua sits on a geothermal field, which has continuous ambient low-level H_2S and mercury.	Elevated standardized incidence ratios were found diseases of the respiratory system, particularly in Maori women (SIR 1.01; 95% CI 0.99 -1.04). Statistically significant increases in the standardized incidence ratio for 'other diseases of the upper respiratory tract' was found (SIR 1.27; 95%CI 1.20 - 1.33) and statistically significant decreased SIR for acute respiratory infections were observed (SIR 0.88; 95% CI 0.83 - 0.93).
Bomans et al. (1997) Case reports of acute H_2S poisoning.	Four out of 5 poisoning victims followed up 8 - 22 months after had pulmonary sequelae consisting of mild restrictive impairment, cough, bronchorrhea and bronchial hyperreactivity, which were severe and persistent or moderate and transient. The findings indicate that pulmonary sequelae include reactive airways dysfunction syndrome.
Hessel et al. (1997) Cross-sectional study of 176 full-time workers in the oil and gas sector. Questionnaires were administered and lung function tests undertaken.	After controlling for age and cigarette use, workers who experienced a 'knockdown' were at statistically significant increased risk for shortness of breath while hurrying on the level or up a small hill (OR 3.6; 95% CI 1.02 - 12.4), wheeze with chest tightness (OR 5.2; 95% CI 1.29 - 20.6) and attacks of wheeze (OR 5.1; 95% CI 1.28 - 20.6) compared to controls. Increased risks were found, although not statistically significant, for the following symptoms: woken by cough, shortness of breath, and wheeze apart from colds. No difference between the exposure and control groups was found for the lung function indicators FEV1, FVC, and the ratio between the two parameters. In workers exposed to sour gas ' that was so strong that it caused symptoms', increased risks for shortness of breath after exercise and attacks of wheeze were found, although they were not statistically significant.
Thu et al. (1997) An Iowa study compared physical and mental health symptoms among people residing within a 2-mile radius of a 4000-head swine operation and a demographically similar control group, which did not have intensive livestock activities.	Those who lived in the vicinity of the intensive hog operation reported higher frequencies of 14 of 18 physical health symptoms, especially respiratory symptoms.

Bhambhani et al. (1996) Men and women volunteers inhaling 10 ppm H_2S for 15 minutes.	No effect on pulmonary function tests was observed.
Partti-Pellinen et al. (1996) Cross-sectional study of 336 adults living near a pulp mill and of 380 adults in a reference community for both 4-wk and 12-mon prior periods. In the exposed community, the annual mean TRS and SO_2 concentrations measured were 2-3 ug/m3 and 1 ug/m3, respectively. In the reference community, the SO_2 concentration was 1 ug/m3.	After controlling for confounders, compared to the reference community, increased risks for headache or migraine in the previous 4 weeks (OR 1.83; 95% CI 1.06 - 31.5) or past 12 months (OR 1.70; 95% CI 1.05 - 2.73), cough in the past 12 months (OR 1.64; 95% CI 1.01 - 2.64), and respiratory infection were found. Increased risks for nasal symptoms were also found but only for the previous 4-week period. This study has found adverse health effects associated with lower concentrations than reported previously.
Bhambhani et al. (1996) Nine men and 10 women inhaled medical air or 10 ppm H_2S for 15 minutes while exercising at 50% at their maximal aerobic power. Pulmonary function tests were done at rest and immediately after the exposures.	No significant changes were found for any of the lung function test parameters (flow volume loop, maximum ventilation volume, lung diffusion capacity). No signs or symptoms were experienced.
Cormier et al. (1996) A case report of a man who developed reactive airways dysfunction (RADS) after exposure to gases in a swine confinement building.	After the worker had started a pit pump to agitate swine manure to turn it into a slurry for easier removal, at the time the ventilator above the pit was not functioning, he noticed higher than usual repugnant odour and his animals falling unconscious. He became dizzy and short of breath and barely managed to get outdoors. At the hospital, his clinical exam was unremarkable except for labored breathing. One month later, his lung function tests showed a mild obstructive defect and positive methacholine challenge. Six and 18 months after the acute exposure, repeat provocations showed persistent increased airway responsiveness.
Trubnikov et al. (1996) A summary of 32 cases of intoxication varying in severity caused by inhalation of sulphur-containing natural gas of Astrakhan gas deposit. Experimental data was obtained by poisoning rats with the gas.	Pneumopathies (toxic pulmonary edema, pneumonitis, asthmatic bronchitis, alveolitis) appeared to play a central role. Mechanisms of the pneumopathies were traced to morphologic disorders of blood-lung barrier, compromised function of lung surfactant, disturbances in lipid peroxidation and anaerobic glycolysis.

Marttila et al. (1995) A small population living near a pulp mill was surveyed. A baseline questionnaire administered to 81 adults was followed by 6 consecutive questionnaires after 3 pre-defined exposure levels (daily mean total reduced S < 10, 10 - 30, > 30 ug/m3).	A dose-related increase in nasal (stuffy or runny nose) and pharyngeal irritation was found. For nasal symptoms, the risk was increased 3.13 (95% CI 1.25 - 7.25) for medium exposure and increased to 8.50 (95% CI 3.19 - 18.64) for high exposure. For pharyngeal symptoms, the risk was increased 2.0 fold (95% CI 0.92 - 4.14) and 5.20 fold (95% CI 1.95 - 10.99) for the median and high exposures, respectively. The medium exposures during the study were reported as daily mean air concentrations (ug/m3) as follows: TRS 11 - 14; SO_2 4 - 16; TSP 45 - 67, NOx 16-23. At about 10 ug/m3 TRS, increased risks were observed for nasal and pharyngeal symptoms.
Richardson (1995) Cross-sectional study of 68 sewer workers and 60 water treatment workers. Lung function tests were done. Workers exposure was categorized by job title.	A significant increased prevalence of obstructive lung disease (OR 21.0, 95% CI = 2.4 - 237.8) and a dose related response was observed in non-smoking sewer workers compared to non-smoking water treatment workers. A significant reduction in respiratory function test parameters was also observed in the sewer workers compared to the water treatment workers. These effects were attributed to long term hydrogen sulphide exposure, however an alternative explanation for the findings may be due to the endotoxin exposure, known to produce lung inflammation, that sewer workers experience to a greater degree than water treatment workers.
Anonymous (1995) Investigators of the National Institute for Occupational Safety and Health conducted a health hazard evaluation at a wastewater treatment plant in Independence, Missouri.	Employees reported sore throats, unexplained coughing accompanied by hoarseness and other complaints while working in the belt pressroom. Measurements of personal breathing zone concentrations of H_2S were: maximum 10-minute ranged from 0.1 ppm to 95 ppm; 8 of the 13 personal breathing zone samples exceeded 10 ppm and 3 exceeded 20 ppm. Maximum 10-min concentrations in general air samples obtained in the belt pressroom range from 46 - 69 ppm whereas outside the belt pressroom, the maximum 10-min concentrations ranged from non-detectable to 0.1 ppm.
Marttila et al. (1994a) Indoor and outdoor concentrations were simultaneously measured with SO_2 analyzers. The filtering effect of three different materials was also tested.	Ambient air sulphur compounds penetrate effectively indoors after some delay and the concentrations decrease much slower indoors than outdoors. People living near pulp mills are not protected from ambient air TRS when indoors and they may be more severely exposed. Their exposure depends on emissions, wind direction, as well as the ventilation system of the building. An aluminum oxide filter improved the indoor air quality.

Marttila et al. (1994b) The effects of long-term exposure to malodorous sulphur compounds on 134 children's' respiratory health were assessed by administering a questionnaire and comparing the occurrence of symptoms in variously polluted areas.	Compared to the non-polluted area, for exposures to polluted air during the previous 4 weeks, increased symptoms of cough [OR 1.83; 95%CI 0.75 - 4.45] and nasal symptoms [OR 1.40; 95%CI 0.59 - 3.31] in children were found. For exposure during the previous 12 months, increased cough [OR 2.28; CI 0.95 - 5.47], nasal symptoms [OR 2.47; CI 0.93 - 6.53] and headache [OR 1.77; CI 0.69 - 4.54] were also found. The estimated and measured concentrations of malodorous sulphur compounds are in Table 7.14.2.
Rossi et al. (1993) A study of the relationship between pollutant levels and emergency treatment for respiratory disease in an industrial town in northern Finland. The number of daily attendances for asthma attacks at the emergency room of Oulu University Central Hospital was recorded over one year together with daily meteorological readings, air pollution levels and pollen counts.	Increased levels of pollutants (NO_2 mostly, but also SO_2, total suspended particulates, and H_2S) were associated with increased attacks of asthma for which emergency treatment was sought. During the year, the daily mean pollutant concentrations (mean and range in ug/m3, respectively) were as follows: SO_2 10, 0 - 56; H_2S 3.1; 0 - 34; NO_2 13.4; 0 - 69; TSP 18.3; 0 - 90.
Haahtela et al. (1992) Subjects living downwind of a paper mill that released hydrogen sulphide and mesityloxide for two days, The 24-hr. average H_2S concentration for the two days was 35 and 43 ug/m3; a 4-hr. maximum was 135 ug/m3; mesityloxide was not measured.	Significantly increased symptoms of difficulty breathing in 33% and increased mental symptoms (depression and anxiety) in 10% of 75 subjects living downwind of a paper mill. Sixty-three percent of respondents reported at least one symptom following the release compared to 26% during the reference period, 4 months later during a low-exposure period. Eye symptoms, cough or pharyngeal irritation, breathlessness, nausea and headache were experienced more often during the release period than the reference period.

Parra et al. (1991) Case report of a worker who, after entering the toilet facilities of his workplace, had acute suspected H_2S poisoning. A new type of pulmonary injury - H_2S inhalation appeared as a subacute illness that develops into chronic functional disability.	14 men were exposed to gases from toilet facilities. One of the exposed patients reported eye, nose and throat irritation when he entered the toilet facility, was free of symptoms until 3 wks after the incident. He began to notice shortness of breath, chest tightness and haemoptysis. Chest X-ray showed a mild bilateral interstitial pattern. Bronchoscopy showed a reddish mucosa throughout the bronchial tree. Bronchoalveolar lavage showed 98% macrophages, 1% neutrophils, and 1% lymphocytes. Pulmonary function tests showed a mild restrictive disease. After 5 months, the patient had residual exertion dyspnea, and lung volumes and CO diffusing capacity were still low. Of the thirteen workers that entered the toilet facility, three were admitted to hospital because of nausea, vomiting, dizziness and shortness of breath; one died. Only one of the workers noticed a rotten egg odour; and ten other workers reported nausea, vomiting, itchy eyes, and nose irritation that recovered without complications after a few hours.
Jaakkola et al. (1991) The frequency of upper respiratory tract infections in children over a 12-month period in a city with pulp mills and chemical plants were compared with two reference cities in Northern Finland.	After controlling for potential confounders, compared with the reference cities, both young (OR 2.0; 95% CI 1.3 - 3.2) and older children (OR 1.6; 95% CI 1.1 - 2.1) had more upper respiratory tract infections during the previous 12-months than the reference cities. Industrial emissions of nitrogen oxides, hydrogen sulphide and methyl mercaptan were the main qualitative differences between the polluted and reference cities. In the polluted city, the annual mean concentration of H_2S was 2.3 ug/m3, SO_2 13-23 ug/m3, NOx 15 ug/m3, and particulates 31 ug/m3.
Bhambhani and Singh (1991) Men were exposed to 2 and 5 ppm H_2S for more than 16 minutes after graded exercise that was performed to exhaustion.	No effects on expired ventilation or maximum power output were found. At 2 and 5 ppm, the respiratory exchange ratio was decreased significantly compared to controls.
Wingren et al. (1991) A case-referent study of 4070 pulp and paper mill workers in Sweden was undertaken.	A significantly increased mortality was seen for diabetes mellitus and for secondary tumors of the lung and liver among the pulp and paper mill workers. Indications of excess risks were also found for obstructive lung disorders, pulmonary emboli, accidents and pneumonia, as well as for malignant lymphomas, leukemia, and cancers of the pancreas and stomach. In the only parish where only the sulfite process was used, digestive tract cancer, especially of the rectum, was found in excess.

Jappinen et al. (1990) Respiratory function was assessed on 26 male pulp and paper workers before and after exposure to H_2S concentrations usually below 10 ppm. Similarly, respiratory function of 10 asthmatic volunteers was assessed before and after exposure to 2 ppm H_2S for 30 min.	No significant changes between a pre- and post-workshift respiratory function and bronchial reactivity to methacholine challenge in the workers were found. On average, ten asthmatic volunteers had a 26% increase in airway resistance and an 8% decrease in specific airway conductance following exposure to 2 ppm H_2S for 30 minutes in an exposure chamber. Two out of ten asthma volunteers exposed had signs of bronchial obstruction.
Jaakkola et al. (1990) Questionnaires were administered twice in a cross-sectional study of air pollution health effects in three communities surrounding paper mills in South Karelia, Finland, involving 488 adults. Monitoring station data and dispersion modeling was used to derive exposure estimates (Table 7.14.2.)	After controlling for potential confounders, compared to a non-polluted community, consistent dose-response relationships were found. Significantly increased risks for eye, nasal symptoms, and cough were found. Moderate to severe exposure increased risk to eyes 11.70 - 11.78 times; increased risk for nasal symptoms 2.01 - 2.19 times; and increased risk for cough 1.89 to 3.06 times, respectively. The symptoms of breathlessness or wheezing and headache were also more frequent in the exposed communities however the differences were not statistically significant. The annual mean H_2S and methyl mercaptan concentration were estimated to be 8 (6 ppb) and 2 - 5 ug/m3 (1 - 2.5 ppb), respectively.

Table 25 Pollutant Concentrations in Study* Airsheds

Pollutant (µg/m3)*	Non- Polluted	Moderately Polluted	Severely Polluted
HYDROGEN SULPHIDE			
- Annual	-	1 (0.7 ppb)	8 (5.7 ppb)
- Daily		15 (10.7 ppb)	100 (71.4 ppb)
Methyl Mercaptan			
- Annual	-	<1	2-5
- Daily	-	20	50
Sulphur Dioxide			
- Annual	-	1	2
- Daily	-	10	10
Particulate			
- Mean		23	31
- Daily Maximum		29	44
Pine Needle Sulphur (µg/g)	964	1400	1800

* Jaakkola et al. (1990) and Marttila et al. (1994); Air concentrations of H_2S were obtained from 4-hr intervals of continuous monitoring. Methyl mercaptan concentration estimates were obtained from dispersion modeling predictions.

Table 26 Respiratory System Effects: Animal Studies

Reference / Study Design	Key Findings
Dorman et al. (1999) Exposed male rats to 0, 10, 30, or 80 ppm H_2S 6 h/day, 7 days/week for 70 days to evaluate the effects of H_2S on nasal epithelium.	Sensory neuron loss and basal cell hyperplasia was observed in the olfactory mucosa following subchronic exposure to 30 or 80 ppm H_2S. Preliminary results suggest that repeated short-term H_2S exposure results in significant inhibition of respiratory and olfactory cytochrome oxidase activity in the nose. The cytochrome oxidase activity of the olfactory epithelium appeared to be inhibited to a greater extent (15 - 20%) than the respiratory epithelium.
Khan et al. (1998a) Fischer 344 rats were repeatedly exposed to 0, 1, 10, and 100 ppm H_2S for 8 hr/d, 5d/wk for 5 wk to study the effects of exposure on various enzyme activities in red blood cells, lungs, liver and brain tissue. At the end of the exposure regimen, the animals were sacrificed and their tissues analyzed.	No changes in enzyme activities were found for the 1-ppm exposure regimen. Significantly lower activities of lung mitochondrial cytochrome c oxidase were observed in animals exposed to 10 and 100 ppm H_2S. In mitochondria isolated from brain, there was a trend toward decreased cytochrome c oxidase activity as the H_2S concentration increased from 1 to 10 and to 100 ppm, however this was not statistically significant. Liver mitochondria cytochrome c oxidase activity did not appear to be affected by the hydrogen sulphide exposures. A small decrease in erythrocyte superoxide dismutase activity (~8%) occurred in rats exposed to 100 ppm H_2S.
Stair et al. (1996) The health of 460 beef cattle and their calves within 3 miles was assessed after a pipeline leak of volatile components of crude sour petroleum, emissions from burning sour condensate and steam washing of gravel occurred at the Red Deer River.	Clinical observations included irritation of mucous membranes, abnormal sexual behavior, decreased bonding of cows with newborn calves and failure of calves to thrive. Some cows had proprioceptive-locomotor deficits attributed to the central nervous system and an increased rate of twinning was present. Histology showed irritation of upper respiratory mucosa represented by nodular and diffuse lymphoid cellular accumulations in the submucosa of the trachea, hyperplasia of tracheal submucosal glands as a response to irritations; and loss of cilia.

Khan et al. (1995) Rats were exposed to sour gas containing 0, 10, 100 and 200 ppm H_2S (duration not specified). Bronchoalveolar lavage (BAL) analyzed immediately after exposure for cellular composition and enzyme activity.	BAL fluid had increased LDH (50-113%) compared to controls. The BAL protein and alkaline phosphatase levels were altered only in the 200-ppm exposure. The 200-ppm exposure also gave a marked increase in neutrophils, and a significant reduction in macrophages in BAL. Also, a marked expansion of the perivascular connective tissue and transudation of proteinaceous (edema) fluid, which was restricted to the walls and perivascular spaces of large arteries, arterioles and venules, was found at the 200-ppm exposure.
Kohno et al. (1991) Male Wistar rats were exposed to 75 ppm H_2S for up to 60 min.	Slight pulmonary congestion was found after 1 hr.
Green et al. (1991) Rats were exposed to 200 and 300 ppm H_2S for 4 hours. Lung lavage fluid surface tension, protein concentrations and LDH activity was measured.	Significant increases in protein and LDH activity were found at both concentrations, however a significant change in lavage fluid surface tension was found only at the high level. Within the alveoli, proteinaceous material and perivascular edema was observed.
Khan et al. (1990) Rats were exposed to various concentrations (10, 50, 200, 400 ppm) of H_2S for 4 hours. The animals were killed immediately, or 1, 24, or 48 hours after exposure and mitochondrial fractions from lung tissue were analyzed for enzyme activity.	No exposure effects were found for succinate-cytochrome c reductase or NADH-cytochrome c reductase. However exposure to 50 ppm and above significantly reduced (25%) lung mitochondrial cytochrome c oxidase activity and exposure to 200 ppm (and above) significantly reduced succinate oxidase activity. In addition, the rate of recovery of cytochrome c oxidase inhibition from exposure was compared for various periods after exposure. For animals exposed for 4 hours at 200 ppm, 1 hour after exposure, cytochrome c oxidase activity remained inhibited at 30% of controls. After 24 hours, inhibition was 12% and after 48 hours, inhibition was 10% indicating a somewhat slow recovery of enzyme activity after exposure.
Green et al. (1990) Rats were exposed to H_2S (500 ppm) with and without capsaicin pretreatment, which depletes the neuropeptide - Substance P, and examined for histopathological lung changes.	20% of the animals exposed to H_2S without capsaicin pretreatment died, whereas 100% of the animals pretreated with capsaicin and exposed to the same concentration died and they died at an earlier time. The capsaicin-pretreated animals also had more severe pulmonary edema with greater concentration of protein in the lavage fluid and heavier lungs postmortem. The airway lesions were similar to those reported for SO_2 and were most severe in the proximal airways with relative sparing of the bronchioles and alveolar ducts.

Table 27 Respiratory System Effects: *In vitro* Studies

Reference / Study Design	Key Findings
Setko (1996) Experiments on animals included acute, subacute and chronic inhalation of natural gas containing hydrogen sulphide at concentrations equal to the maximum allowable concentrations (10 ppm).	The multi-component natural gas was shown to induce more intensive toxic effects than its components. The gas mixture induced greater permeability of cell membranes, compromised biocatalysis systems, increased lipid peroxidation 2-fold, and disturbed thiol-disulfide balance.
Hayden et al. (1990) Human fetal lung cells (WI-38) were grown in monolayers with sodium sulfide and sodium sulfite at concentrations ranging from 0 - 100 uM for 24 hours, after which fresh cell culture medium replaced the test medium. Lactate dehydrogenase (LDH) leakage, total protein and DNA content of cell were measured at various periods after the 24-hour exposure period.	LDH leakage was maximal at 2 hours post exposure to sodium sulfide at all concentrations (0.01, 0.1, 1.0, 10, and 100 uM). At two hours post exposure, the total protein content decreased as the concentration of sodium sulfide increased. These effects were much more severe in the sodium sulfide exposed cells as compared to the sodium sulfite. Incubation of cells with 10 uM of sodium sulfide reduced DNA content to approximately 40% of control values in two days. A similar concentration of sodium sulfite (10 uM) reduced DNA content to 55% of controls 3 days after exposure.

3.0 TOXICOKINETICS: ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

Within the last decade, few published studies were located that examined H_2S toxicokinetics including absorption, distribution, metabolism or elimination. The most recent accounts are given in the ATSDR's Toxicological Profile for Hydrogen Sulfide (1999a) and US EPA Health Assessment Document for Hydrogen Sulfide (1993) reports[28]. Several subsequent reports have provided additional information on the distribution and metabolism. However, no studies were located that examined absorption or elimination.

3.1 DISTRIBUTION

Mitchell et al. (1993) reported that sulfide levels in the kidney, brain, and liver increased 63.7%, 57.2%, and 18.1%, respectively, compared to untreated controls following subcutaneous injection of 60 ug/g sodium sulfide (6 times the LD50) into the interscapular region of mice. Sulfide levels in untreated kidney, liver and brain tissues were 200.1 +\- 46.4, 144.5 +\- 12.4 and 68.9 +\- 11.3 nmol/g wet weight.

Nagata et al. (1990) exposed rats to 550 - 650 ppm H_2S gas in a chamber until death. Sulfide was measured by alkylation by pentafluorobenzyl bromide to form the stable derivative bis(pentafluorobenzyl)sulfide and analyzed by gas chromatography with electron capture detection, giving detection limits of 0.01 ug/g. Lung, brain, thigh muscle, abdominal muscle, liver and kidney tissues were collected immediately after death and the mean values of sulfide concentration were 0.60, 0.31, 0.21, 0.22, 1.67 and 1.45 ug/g, respectively. The sulfide concentration in the blood averaged 0.48 ug/g. Sulfide levels of unexposed rats were non-detectable for lung, brain, thigh muscle and abdominal muscle whereas for liver was 0.95 ug/g and kidney 1.19 ug/g.

3.2 METABOLISM

For the first time, Yong (2001) has shown a linkage of sulfide oxidation to ATP synthesis in an organism not specifically adapted to a sulfide-rich environment. In chicken liver mitochondria, oxygen is consumed at an accelerated rate when supplied with low concentrations of H_2S. Sulfide oxidation was coupled to ATP synthesis and at low concentrations of sulfide presumably close to physiological, the oxygen/sulfide ratio was 0.75. A recent study of fission yeast has identified a gene encoding a mitochondrial enzyme that can oxidize sulfide with sequence homology to sulfide-oxidizing enzymes of bacterial photosynthesis and potential homologs in many other organisms including humans (Vande Weghe & Ow, 1999).

Suarez et al. (1998) found that H_2S production in the rat colon was dependent on dietary components as shown by a 6-fold reduction with fasting and a 5-fold increase with ingestion of carrageenan a nonabsorbable sulfur compound. Zinc acetate reduced cecal H_2S 5-fold, showing the importance of H_2S binding by divalent cations. These investigators also found more than

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[28] These reports are available at the following websites: <http://www.atsdr.cdc.gov/atsdrhome.html>, <http://www.epa.gov/ncepihom/Catalog/EPA600886026F.html>

90% of the sulfur gases were absorbed or metabolized that during passage from the cecum to the rectum. H_2S turnover rate of 97%/min was measured in the isolated cecum, which was 10-fold greater than the accumulation rate. The cecal mucosal tissue metabolized H_2S and methyl mercaptan very rapidly via a non-methylating reaction.

Levitt et al. (1999) reported that H_2S and methyl mercaptan are not detoxified by methylation to dimethylsulfide by rat cecal mucosa homogenates. Instead, methyl mercaptan is demethylated to H_2S, which is converted to nonvolatile metabolites, primarily thiosulfate. Further, analysis of cecal venous blood obtained immediately after intracecal instillation of radiolabelled H_2S revealed that virtually all absorbed H_2S was oxidized to thiosulfate. The oxidation rate was 10,000 times greater than the reported methylation rate.

4.0 RESPIRATION - ANTIOXIDANT ENZYME SYSTEM

Hydrogen sulfide, via several mechanisms, produces an imbalance in the oxidant-anti-oxidant system. There are three reasons to consider the effects of H_2S on anti-oxidant enzyme systems; inhibition of cytochrome c oxidase stops electron transport within the chain thereby generating free radicals from upstream electron transfer proteins; H_2S frees iron from intracellular ferritin stores which then act as catalysts for free radical generation; and H_2S inhibits several of the anti-oxidant enzymes which normally inhibit the damaging effects of free radicals. Enzymes located within the mitochondria (electron transport chain proteins, monoamine oxidase), the endoplasmic reticulum (cytochromes P450), the cytoplasm (xanthine oxidase), and plasma membrane (phospholipase A2 activation, NAD(P)H oxidases) and peroxisomes are all sources of reactive oxygen species. Evidence of anti-oxidant enzyme inhibition or production of oxidants or byproducts was reported by several investigators (Table 4.1) (Boev et al., 1998; Setko, 1996; Trubnikov et al., 1996; Boev et al., 1992; Beck, 1992; Church, 1992) and in earlier studies (Table A20, Appendix I; Haider et al., 1980; Haider and Hasan; 1984).

The main anti-oxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase and catalase. These enzymes are all highly conserved, found in all eukaroytic cells, and are involved in the mechanisms of cellular defense against oxidative damage. Fully functional antioxidant enzymes have greater importance under oxidative stress conditions, which occurs following electron transport chain inhibition.

Inhibition of the cytochrome c oxidase complex by sulfide may produce oxygen radicals as well as sulfide radicals, which could act as initiators of peroxidation. The generation of sulfide radicals has been suggested based on the spectral data (Nicholls, 1975, 1982). Sulfide has also been reported to inhibit CuZnSOD (Khan et al., 1988), which would be expected to lead to an over-production of oxygen radicals (Halliwell, 1999).

4.1 SUPEROXIDE DISMUTASE

Cu,Zn Superoxide dismutases[29] (SOD), found in the cytoplasm of all eukaryotic cells, are homodimers joined by a disulfide bond. Earlier studies by Khan et al. (1987) exposed bovine red blood cells to various concentrations of sulfide, sulfite, and sulfate *in vitro* to determine their effects on various enzymes. SOD activity was 78.7% of control in the presence of 1.25 mM sodium sulfide; enzyme activity decreased with an increase in concentration. The IC_{50} was approximately 10 mM for sodium sulfide, and just under 20 mM for sodium sulfite. Khan et al. (1998) also exposed rats to various concentrations (0, 10, and 100 ppm) H_2S for 8 hours per day for 5 days/wk for 5 weeks. At the end of the exposure regimen, SOD activity was determined in the red blood cells, and lung, brain and liver mitochondria. No significant changes were observed in red blood cells, however there was a slight reduction trend although not statistically significant, corresponding with increased exposure in other tissues. These findings may be due to differences between *in vivo* and *in vitro* concentrations.

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[29] Cu,Zn-Superoxide dismutase (SOD), which is present in all oxygen-metabolizing cells is a cytoplasmic and extracellular enzyme consisting of two subunits, each containing one Cu and one Zn atom, joined by a disulfide bond (Battistoni, 1998). Zinc is believed to have a stabilizing structural role and the copper is directly involved in the catalytic activity (Forman and Fridovich, 1973). The rate of catalysis is very fast, about 2-3 X 10^9 L /mol.sec, is diffusion limited and is enhanced by the electostatic guidance of the substrate to the active site, near a copper in a deep pocket of the subunit (Battistoni, 1998; Messerschmidt, 1998)

Florence (1996) reported reductions of erythrocyte SOD activity in 8/10 samples collected from cattle in 10 herds during the Lodgepole blowout compared to samples collected 1 year later.

4.2 GLUTATHIONE PEROXIDASE AND REDUCTASE

In mitochondria, glutathione peroxidase uses glutathione to catalyze the reduction of hydrogen peroxide and other peroxides to water. In most tissues, glutathione is present intracellularly in millimolar concentrations. Glutathione reductase then regenerates the oxidized glutathione. However with a disulfide bridge at the reductase active site that is necessary for electron transfer (Halliwell, 1998, p 144), H_2S would be expected to inhibit its activity. This has been shown to be the case in bovine erythrocytes (Khan et al., 1987).

4.3 CATALASE

Although exposure concentrations were not available, a depression of catalase in cerebral cortex of rats exposed to gas condensate containing H_2S was reported by Boev et al. (1992). Catalase, a peroxisomal tetrameric heme protein catalyzes the dismutation of hydrogen peroxide that was produced by superoxide dismutase, into water and molecular oxygen at an enormous rate (Halliwell, 1998, p 137). Dissociation of catalase into its subunits, which occurs readily on exposure to acid, causes loss of activity (Halliwell, 1998, p 136). It is reportedly inhibited (Keilin & Hartree, 1938) which is partly reversible by 8 - 57 μM sulfide (Beers and Sizer, 1954) and by methyl mercaptan (Finkelstein et al., 1986). As very low levels of catalase activity are found in the brain and heart (Halliwell, 1998, p 135), these tissues may be particularly susceptible to the oxidative damage of hydrogen peroxide accumulated as a consequence of H_2S inhibition.

The importance of compartmentalized reactions needs to be emphasized. Glutathione (GSH), in the reduced form, serves to keep cysteine residues in cytoplasmic and endoplasmic reticulum proteins in the reduced state and to detoxify hydrogen peroxide and organic peroxides. It is present in these compartments at about 500 times greater than oxidized glutathione (GSSH). H_2S inside the cytosol would be expected to reduce GSSH to GSH and perhaps, if in large excess, form persulfides or polysulfides (Toohey, 1989). While there are other anti-oxidant enzymes such as superoxide dismutase and catalase in the cytoplasm, it is inside the mitochondria where most of the reactive oxygen species are generated that need to be neutralized by anti-oxidants. In the mitochondria, superoxide dismutase is available to convert superoxide to hydrogen peroxide and glutathione peroxidase is available to convert catalase to water. However, as noted above these enzymes are inhibited to varying degrees by hydrogen sulfide. Further, the mitochondria do not appear to be able to export the oxidized glutathione out of the organelle and that it must be reduced *in situ*. This is believed to be why the loss of mitochondrial GSH rather than cytosolic GSH is critical in some types of cell injury (Reed, 1990).

Table 28 Effects on Anti-oxidant Enzyme Systems

Boev et al. (1998) Chronic action of substances as constituents of condensed gas was studied. The concentration of corresponded to the maximum acceptable air concentration.	Changes in the activity of xenobiotic enzymes, suggestive of damage to the hepatic microsomal monooxygenase system, were found. As biotransformation rates increased, microsomal monooxygenase activity increased, as did the generation of active oxygen forms and hydrogen peroxide.
Setko (1996) Experiments on animals included acute, subacute and chronic inhalation of natural gas containing hydrogen sulphide at concentrations equal to the maximum allowable concentrations (10 ppm).	The multi-component natural gas was shown to induce more intensive toxic effects than its components. The gas mixture induced greater permeability of cell membranes, compromised biocatalysis systems, increased lipid peroxidation 2-fold, and disturbed thiol-disulfide balance.
Trubnikov et al. (1996) A summary of 32 cases of intoxication varying in severity caused by inhalation of sulphur-containing natural gas of Astrakhan gas deposit. Experimental data was obtained by poisoning rats with the gas.	Pneumopathies (toxic pulmonary edema, pneumonitis, asthmatic bronchitis, alveolitis) appeared to play a central role. Mechanisms of the pneumopathies were traced to morphologic disorders of blood-lung barrier, compromised function of lung surfactant, disturbances in lipid peroxidation and anaerobic glycolysis.
Boev et al. (1992) Wistar rats were exposed to gas condensate containing H_2S in an open field (specifics were not available)	Surplus accumulation in the cerebral cortex tissue of products of peroxide lipid oxidation and depression of catalase was reported.
Beers and Sizer (1954) *In vitro* studies of sulfide's effect on beef liver catalase were undertaken.	8- 57 uM sulfide inhibited beef liver catalase and the inhibition were only partly reversible.

Excess sulfide may lead to disruption of the iron sulphur clusters (Beinert et al., 1997), releasing iron, which is known (especially ferrous iron) to be a potent free radical generator in brain tissue (Rauhala and Chiueh, 2000).

In addition to the ability of sulfide to inhibit cytochrome oxidase c, many other enzymes involved in energy production are also susceptible. Enzymes containing Fe-S clusters are susceptible to inactivation or inhibition by excess sulfide or reactive oxygen species (Beinert et al., 1997). In the TCA cycle, succinate dehydrogenase and NADH dehydrogenase have Fe-S clusters and in the electron transport chain, all of the complexes with the exception of cytochrome c oxidase, have Fe-S clusters: 6 or 7 in Complex I, 1 [4Fe-4S] and 2 [2Fe-2S] clusters in Complex II, and 1 [2Fe-2S] in Complex III. Energy deprivation in the neuron leads to relief of the voltage-dependent Mg2+-block of the NMDA receptor channel, thereby enabling the excitatory amino acids to stimulate persistently at the receptor and produce neuronal cell death (Novelli et al., 1988; Henneberry et al., 1989).

The mechanism of cytochrome c oxidase inhibition is believed to be due to reduction and binding of CuB and cytochrome a3 by sulphide, making re-oxidation difficult (Hill et al., 1984). However, in some studies using intact mitochondria and whole cells, continued respiration has been reported at concentrations of 100 micromolar HS- (Bartholomew, 1980 [cited in Searcy; 1995]). Recent work by Davey et al. (1998) indicates that Complex I is the rate-limiting step in the chain and most likely to be the main regulator of oxidative phosphorylation. These investigators examined the relative contribution of individual mitochondrial chain complexes to the control of NAD-linked substrate oxidative phosphorylation in synaptic mitochondria. They found that before major changes in the rates of oxygen consumption and ATP synthesis occurred, Complex I, III, and IV activities were decreased by 25, 80 and 70% respectively. Given the susceptibility of Fe-S clusters to high sulfide concentrations (Beinert et al., 1997) and that Complex I contains 6 to 7 iron-sulphur clusters that participate in the electron transfer process (Voet, 1999), it is possible that H_2S inhibits respiration by disrupting Fe-S clusters directly or indirectly due to reactive oxygen species generation via cytochrome c oxidase inhibition.

5.0 CUMULATIVE HEALTH EFFECTS OF HYDROGEN SULFIDE

Many examples of cumulative health effects of repeated low-level H_2S exposure exist, which does not support earlier claims that H_2S is only an acute toxicant due to its rapid metabolism to non-toxic products. The observed cumulative effects are likely consequences of several interacting factors or processes. At the organism level, factors such as gender, age, genetics, nutritional status, co-exposures to other toxic substances, and control over environment, are relevant. At the tissue or organ level, the factors include; disruption of mucosal layers; chronic irritation or inflammation; altered immune defense, and altered tissue repair processes. At the cellular level, these factors include: accessibility of protein to H_2S and its anions; the binding affinity of H_2S (or its anions) to enzymes and metalloproteins[30]; and the half-life or time to reassemble or synthesize affected proteins in the tissues[31]. Thus, it is worth noting that after studying acute and subacute poisonings experimentally, earlier reviewers reported that harmful after-effects of H_2S exposure were more common in cases of severe subacute than severe acute poisoning (Sayers et al., 1923).

5.1 CARDIOVASCULAR SYSTEM

Studies conducted during the past decade have provided evidence suggestive of a causal association between long-term exposure to H_2S and development of cardiovascular disease. Consistent increases in risk for cardiovascular disease were found in several large epidemiological studies of occupationally (Gamble et al., 2000; Lewis et al., 2000; Betemps et al., 1994; Hammar et al., 1992; Ahlman et al., 1991Jappinen and Tola, 1990; Jappinen, 1987; Milham and Demers, 1984) and environmentally exposed groups (Bates et al., 1998). These findings are supported by the case reports of acutely exposed workers (Schneider et al., 1998; Gregorakos et al., 1995; Tvedt et al., 1991a) that report cardiac changes such as sinus tachycardia, ischemic changes, and elevated cardiac enzymes (Gregorakos et al., 1995; Tanaka et al., 1999), particularly CK-MB, which is diagnostic of myocardial infarction.

5.2 RESPIRATORY SYSTEM

5.2.1 Children - Community Studies

Long-term exposure to low levels of malodorous sulfur compounds (annual average H_2S 0.7 - 5.7 ppb; daily average 11 - 71 ppb) is associated with increased cough and nasal symptoms (Marttila et al., 1994b), and upper respiratory tract infections (Jaakkola et al., 1991), supporting earlier findings (Holasova, 1969). Children living near a natural gas refinery have been reported to be at increased risk for persistent cough, persistent phlegm, wheezing and to have reduced pulmonary function associated with exposure (Boev et al., 1998), supporting similar findings from earlier studies (Dales et al., 1984; 1989 [Table A24; Appendix I]).

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[30] The solubility product constant (Ksp), the equilibrium constants for the dissolution of slightly soluble solids in water with dissociation into their aqueous ions, provide an approximate measure of the sulfide's binding affinity to the respective metal. The findings of prolonged cytochrome c oxidase inhibition by several investigators (Salvolainen, 1980; Khan, 1999) are consistent and confirm earlier findings by Nicholls (1975) that the sulfide-cytochrome aa3 complex dissociates slowly.

[31] For example, the estimated half-life of rat liver cytochrome c is 150 hours and cytochrome b 130 hours (Voet, 1999, p 612). If these and similarly long-lived proteins are not re-synthesized, or repaired, altered function and cellular impairment may develop. Further, proteins containing disulfide bonds may be improperly formed as disulfide bond formation is linked to the electron transport chain (Bader et al., 1999).

5.2.2 Adults - Community Studies

Bates et al. (1998) found a statistically significant increase in 'other diseases of the upper respiratory tract' in residents of Rotorua, New Zealand, compared to the rest of the country. Exposure to total reduced sulfur compounds (annual mean total reduced sulfur concentrations measured were 2-3 ug/m3) near a pulp mill was associated with an increased cough, nasal symptoms, and respiratory infections (Partti-Pellinen et al. (1996). In a study involving three communities surrounding paper mills in South Karelia, Finland, Jaakkola et al. (1990) found consistent dose-response relationships and significantly increased risks for eye, nasal symptoms, and cough. Moderate to severe exposure increased risk to eyes 11.70 to 11.78 times; increased risk for nasal symptoms 2.0 to 2.19 times; and increased risk for cough 1.89 to 3.06 times. The symptoms of breathlessness or wheezing and headache were also more frequent in the exposed communities however the differences were not statistically significant. These findings are in agreement with those of Marttila et al. (1995) and Partti-Pellinen (1996). Marttila et al. (1995) reported a dose-related increase in nasal (stuffy or runny nose) and pharyngeal irritation. For nasal symptoms, the risk was increased 3.13 to 8.5 fold for medium and high exposure respectively (daily mean total reduced sulfur < 10, 10 - 30, > 30 ug/m3 for control, medium and high exposures respectively). For pharyngeal symptoms, the risk was increased 2.0 and 5.2 fold for the medium and high exposures, respectively. Partti-Pellinen (1996) reported an excess of cough and respiratory infection in a community exposed to total reduced sulfur compounds at an annual mean concentration of 2 - 3 ug/m^3. These studies support earlier work by Spitzer et al. (1989), who found an increased proportion of people living near a natural gas refinery had hay fever or allergic rhinitis, nasal symptoms and shortness of breath.

5.2.3 Adults - Worker Studies

Cross sectional studies of workers exposed to H_2S found increased symptoms (Hessel et al., 1997), alterations in respiratory function (Salano and Copello, 1998; Nethercott and Holness; 1988) and increased risk of obstructive lung disease (Richardson, 1995). In a cross-sectional study of 68 sewer workers and 60 water treatment workers, Richardson (1995) found a significant increased prevalence of obstructive lung disease (OR 21.0, 95% CI = 2.4 - 237.8). A dose-related response was observed in non-smoking sewer workers compared to non-smoking water treatment workers. A significant reduction in respiratory function test parameters was also observed in the sewer workers compared to the water treatment workers. In a cross-sectional epidemiologic study of a group of workers employed in sewer network maintenance and urban wastewater treatment plants, the relative risk of alterations in respiratory function (both instrumental and clinical findings) was increased among the water treatment workers (Salano and Copello; 1998). Lung function was assessed in fifty sewage workers using a heat treatment sewage sludge process (Nethercott and Holness; 1988). Workers reported 'influenza-like' symptoms, cough, sputum production, wheeze, sore throat and skin complaints. These workers tended to have reduced lung function, particularly in the area of the plant where the dried sludge was incinerated. In a cross-sectional study of 176 full-time workers in the oil and gas sector, workers exposed to sour gas 'that was so strong that it caused symptoms', Hessel et al. (1997) found increased risks, although not statistically significant, for shortness of breath after exercise and attacks of wheeze.

Acutely H_2S exposed workers also tended to have subsequent alterations in respiratory function (Buick et al., 2000; Van Aalst et al., 2000; Hessel & Melenka, 1999).

5.3 NERVOUS SYSTEM

Few studies addressing the cumulative effects of H_2S in the nervous system have been undertaken in the past decade.

Increased nervous system symptoms were reported in a cross-sectional study of citizens living near an industrial wastewater treatment pond and geothermal processing facility (Legator et al., 2001). Partti-Pellinen et al. (1996) reported an increased risk of headache or migraine in a community exposed to total reduced sulfur (TRS) compounds at an annual mean concentration of 2-3 $\mu g/m^3$. Boev et al. (1998) found that the agents emitted by a sulphide-containing gas processing plant had adverse effects on the functional status of children who resided in the vicinity of the plant, namely, decreased vital capacity of the lung, mental performance, retarded sensory-motor responses, altered enzymatic system.

Persistent headaches were described in case reports of H_2S exposed workers (Hessel & Melenka, 1999; TNRCC, 1998), and citizens (Anonymous, 1996).

In three groups exposed repeatedly to low levels of H_2S, Kilburn et al. (1998) found abnormal test results for simple and choice reaction time, balance, colour vision, trail making, immediate story recall, and digit symbol in group 1; abnormal test results were found for choice reaction time, and balance in group 2 and abnormal test results were found for balance in group 3. Group 1 consisted of 13 former Unocal workers and 22 downwind residents of Unocal refinery; Group 2 was comprised of residents of two communities with oil refineries and the third group consisted of refinery workers evaluated for asbestos exposure

Kilburn and Warsaw (1995) studied six domains of brain function (neuro-physiological, recall, overlearned memory, cognitive, perceptual motor speed and affective) of 13 former workers (none had been overcome by H_2S) and 22 neighbors of a sour crude oil refinery living within 1200 meters of the plant were compared to 32 age-, gender- and education-matched controls. A questionnaire was administered to assess frequency of irritative, respiratory and neurological symptoms. H_2S, measured at street level for 1 week, was 10 ppb with periodic peaks of 100 ppb. Dimethylsulfide was 4 ppb, mercaptans were 2 ppb, and ethane was 500 ppb and propane, 500 ppb. 24-hr averages of gases monitored outside the desulphurization unit were 0.1 - 21.1 ppm mercaptans, 0 - 8.8 ppm H_2S, 2.6 - 52.1 ppm COS, and 6.1 - 70.1 ug/m^3 total reduced sulfur. Worker exposures were not measured. The exposed subjects' mean values were significantly different from controls for two-choice reaction time, balance (as speed of sway), colour discrimination, digit symbol, trail-making A and B, and immediate story recall. Visual recall was significantly impaired in neighbors but not in the ex-worker group. Anger, confusion, depression, tension-anxiety and fatigue scores were significantly elevated in neighbors and former worker groups compared to controls. Respiratory, mucous membrane irritation, neurological symptoms, sleep disturbances, and skin complaints were all more frequent in exposed subjects, compared to controls. Excluding subjects with other chemical exposures (surgical anesthesia, solvents, vibrating tools, seizure medication, pesticide, alcohol) did not affect the results significantly. These findings are in agreement with Wasch and colleague's case reports (1989). Patients exposed to H_2S in separate unrelated incidents showed delayed P-300 event-related potentials. Persistent neurological symptoms and neuropsychological testing indicated that all three patients developed persistent cognitive impairment (Wasch et al., 1989).

Few studies were undertaken in the past decade that addressed potential cumulative impacts of H_2S exposure. Several of the animal studies suggest adverse nervous system effects following repeated exposures to H_2S. Sensory neuron loss and basal cell hyperplasia were observed in the olfactory mucosa following subchronic exposure of male rats to 30 or 80 ppm H_2S 6 hr/day, 7 days/wk for 70 days (Dorman et al., 1999). After rats were repeatedly exposed to 125 ppm H_2S, their ability to learn and relearn new tasks, evaluated using a 16-arm maze, was altered (Partlo et al., 1998). Rat hippocampal theta activity was recorded for 10 minutes just prior to H_2S exposure and during the final 10 minutes of exposure (25, 50, 75 or 100 ppm H_2S for 3 hr/day for 5 consecutive days) (Skrajny et al., 1996). The total power of hippocampal theta activity increased in a concentration-dependent manner in both the dentate gyrus and CA1 regions of the brain. Repeated exposures for 5 consecutive days resulted in a cumulative effect that required 2 weeks for complete recovery. In earlier studies, guinea pigs, exposed to 20 ppm H_2S for one hour per day for 11 days, showed signs such as fatigue, somnolence, dizziness, itching, eye irritation and reduced concentrations of cerebral hemisphere and brainstem total lipids (19, 34%) and phospholipids (11, 21%) (Haider et al., 1980). An 18% increase in malonaldehyde in the central hemisphere was also reported, suggesting coincident lipid peroxidation.

Biochemical changes in animals repeatedly H_2S-exposed were reported by Khan et al. (1998; 1990). Fischer 344 rats were exposed to 0, 1, 10, and 100 ppm H_2S for 8 hr/d, 5days/wk for 5 wk (Khan et al. (1998a). No changes in enzyme activities were found for the 1 ppm exposure regimen, however, significantly lower activities of lung mitochondrial cytochrome c oxidase were observed in animals exposed to 10 and 100 ppm H_2S. In mitochondria isolated from brain, there was a trend toward decreased cytochrome c oxidase activity as the H_2S concentration increased from 1 to 10 and to 100, however this was not statistically significant. Liver mitochondria cytochrome c oxidase activity did not appear to be affected by the H_2S exposures. A small decrease in erythrocyte superoxide dismutase activity (~8%) occurred in rats exposed to 100 ppm H_2S. Rats exposed to various concentrations (10, 50, 200, 400 ppm) of H_2S for 4 hours and killed immediately, or 1, 24, or 48 hours after exposure and mitochondrial fractions from lung tissue were analyzed for enzyme activity (Khan et al., 1990). The rate of recovery of cytochrome c oxidase inhibition from exposure was compared for various periods after exposure. For animals exposed for 4 hours at 200 ppm, 1 hour after exposure, cytochrome c oxidase activity remained inhibited 30%. After 24 hours, inhibition was 12% and after 48 hours, inhibition was 10% indicating a somewhat slow recovery of enzyme activity after exposure. These findings support earlier work by Savolainen et al. (1980) who found that repeated exposures of mice to 100 ppm for 2 hr for 4 days produced significant cumulative decreases in cytochrome oxidase c activity in the brain.

5.4 IS HYDROGEN SULPHIDE A CUMULATIVE POISON?

Citing the work of Baxter et al. (1958), Baxter and Van Reen (1958), Sorbo (1958), MacLeod et al., (1961a,b) Bartholomew et al. (1980) and Curtis et al. (1972), previous literature reviews have stated that H_2S is not a cumulative poison (NIOSH, 1977; US EPA, 1993; ASTDR, 2000), due to its rapid oxidation and excretion. This implies that oxidative processes toward H_2S are instantaneous and complete in all tissues, which is certainly not the case especially in light of its ability to inhibit many oxidative enzymes (Valentine, 1984, Khan, 1989). While metals such as iron or copper may oxidize H_2S, they are more likely to form a metal - sulfide[32], producing a strong bond that could impair enzyme activity if the metal is within an enzyme's active site. Virtually all of the enzymes inhibited by hydrogen sulfide are metalloenzymes or use metals as cofactors in the catalytic mechanism (Table 29).

Further, the high reactivity of H_2S and its anions with proteins, oxygen and water ensures a wide range of products in any system. While the reviewers identified several categories of reactions that H_2S may have in biological systems (oxidation to thiosulfate or sulfate, disulfide bond cleavage, interaction with metalloproteins, and methylation), no mention was made of the reactivity of H_2S toward carbon-carbon or carbon-oxygen double bonds, producing nucleophilic addition products (Friedman, 1994), towards heme-cytochrome thioether linkages[33], thioesters, or towards the sulfonium ion of S-adenosylmethionine. In several reviews, it appeared that the aforementioned reactions were classified as detoxication reactions. Yet toxicological effects can be expected when highly conserved metalloenzymes are inhibited and when protein structures are disrupted by cleavage of their disulfide bonds. This is especially significant due to the linkage between the disulfide bond formation and electron transport chain function.

The studies cited in the above-mentioned reviews attribute H_2S oxidation to various enzymes - sulfide oxidase and sulfite oxidase. Sulfide oxidase, as a separate enzyme, has not been characterized. Oxidase activity against sulfide has not been detected in colonocytes, even though glutathione peroxidase activity is high in these cells (Lash et al., 1986).

Hydrosulfide as a monovalent anion, may actually inhibit electron transfer in sulfite oxidase (Sullivan et al., 1993). Also, due to the coupling of electron transfer to ATP synthesis of both sulfite oxidase (which uses cytochrome c as an electron acceptor) and cytochrome c oxidase (which uses cytochrome c as an electron donator), inhibition effects are expected to be amplified *in vivo*.

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[32] In rats and guinea pigs exposed to an atmosphere containing H_2S for 1 min - 10 hrs, granules that stained with silver made their appearance in neurons and axons, liver cells and bile capillaries, renal tubular cells, and pancreatic acinar and islet cells. The granules apparently represented sulfides of heavy metals (Voigt & Muller, 1955).

[33] While formation of a thioester bond is believed to be transitory and reversible, adducts linked through thioether bonds such as prenyl anchors and protoporphyrin rins are not known to be reversible (Thomas and Mallis, 2001).

Table 29 Structure - Function Summary of Enzymes Susceptible to H_2S Inhibition

Cellular Compartment	Remarks
Cellular Membrane	
▪ Alkaline Phosphatase	Zinc metalloenzyme requires magnesium as a cofactor
▪ Carbonic Anhydrase	Zinc metalloenzyme; hydroxyl group at active site is likely replaced by hydrosulfide anion.
▪ Guanylate cyclase/ G-protein	Thioether linkage inside cell membrane
Cytosol	
▪ Glycolysis	Five of 7 enzymes in the glycolysis pathway are inhibited by H_2S. These include; hexokinase, phosphofructokinase, phosphoglycerate kinase, enolase, and pyruvate kinase; all require magnesium ions as cofactors.
▪ Glycogen synthesis	Glycogen synthetase
▪ Pentose phosphate pathway	G6PD, 6-phosphogluconate dehydrogenase
▪ Heme Synthesis	In addition to aconitase, one of the cytosolic heme synthesis enzymes is reportedly inhibited by H_2S - ALA dehydrase, a zinc metalloenzyme.
▪ Amino Acid Transaminases	Two transaminases are reportedly inhibited by H_2S: glutamic oxaloacetic transaminase and glutamic pyruvic transaminase.
▪ Anti-Oxidant Enzymes	Cu-Zn superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase
Mitochondria	
▪ Citric Acid Cycle	Several enzymes within the citric acid cycle are inhibited by H_2S. These include pyruvate dehydrogenase, citrate synthase and aconitase. Reductions in this cycle will lead to reductions of carbon dioxide, NADH and $FADH_2$.
▪ Electron Transport Chain	Complex IV (cytochrome c oxidase), a complex metalloenzyme containing 3 Cu, 2 Fe (heme), 1 Mg and 1 Zn atoms, of the electron transport chain. Also, Complex V (ATPase).
▪ Fatty Acid Oxidation	Short-chain acyl-CoA dehydrogenase; inhibition by H_2S may be due to inhibition of electron transport chain at complex IV.
▪ Heme Synthesis	Two mitochondrial enzymes in the heme synthesis pathway are inhibited by H_2S. These include delta amino levulinic acid synthase, which is the rate-limiting enzyme, and ferrochelatase.
▪ Anti-Oxidant Enzymes	Manganese superoxide dismutase
▪ Neurotransmitter Enzyme Production	Monoamine oxidase; located in the outer mitochondrial membrane
Peroxisomes	
▪ Catalase	Subunits are joined by disulfide bonds
▪ Amino acid oxidases	

Smooth endoplasmic reticulum	
▪ Lipid biosynthesis	HMGCoA Reductase
Extracellular Compartment ▪ Neutral endopeptidase	Zinc metalloenzyme
▪ Carboxypeptidase	Zinc metalloenzyme
▪ Von Willebrand Factor	? Copper-containing protein
Unknown Compartment ▪ Peptidylglycine alpha Amidating Monooxygenase	Copper metalloenzyme
▪ Peptidylglycine alpha Hydroxylating monooxygenase	Copper metalloenzyme
▪ Renal dipeptidase	? Zinc metalloenzyme

6.0 EXISTING GUIDELINES

The current Alberta Environmental Protection ambient air guideline[34] for H_2S (10 ppb) is based on odour effects. The documentation states "*odour is considered the limiting factor in setting ambient hydrogen sulphide limits. No health or vegetation effects have been reported at the selected ambient levels of* H_2S. *Odour threshold is set at 0.01 ppm concentration of* H_2S. *Threshold Limit Value (TLV) is set at 10 ppm. The minimum concentration of* H_2S *at which human health effects (minor) start is 20 ppm for a short term exposure*". There is no time averaging stated in the guideline's documentation. However, recent Alberta Environmental Protection literature refers to a time-averaging period of 1 hour. For comparison purposes, various Canadian and United States standards and guidelines are given in Tables 6.1 & 6.2. A large inconsistency across jurisdictions is evident as the guidelines or limits vary over 6 orders of magnitude.

Table 30 Summary of Canadian and Other Jurisdiction H_2S Guidelines

Province	Concentration (ppb)	Averaging Time
Alberta	10	1 hr
Ontario*	21	1 hr (health) ambient air quality criteria
	21	30 min (odour) point of impingement standard
Manitoba	1000	1 hr (maximum tolerable)
	11	1 hr (maximum acceptable)
	4	24 hr (maximum acceptable)
	0.7	1 hr (maximum desirable)
Canada	No guideline	
World Health Organization	107 5	24 hr 30 min

* Ontario also has limits for total reduced sulfur: 40 ug/m3; 30 min (odour) point of impingement guideline; and 40 ug/m3 (1 hr) ambient air quality criteria

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[34] The reference given in the guideline is the 1981 National Research Council Committee Report No. 18467 "Hydrogen Sulphide in the Atmospheric Environment· Scientific Criteria for Assessing its Effects on Environmental Quality."

Table 31 Summary of United States H_2S Ambient Air Guidelines (Adapted from: McGavran, 2001)

State	Concentration (ppb)	Averaging Time
Alabama	20,000	30 min
Alaska	35	30 min
Arizona	80	1 hr (welfare)
California	30	1 hr (welfare; nuisance)
Colorado	1	1 hr (welfare)
Delaware	0.04	3 min (health; nuisance)
	30	1 hr
Hawaii	25	1 hr (welfare)
Illinois	10	8 hr (health)
Kentucky	10	1 hr
Louisiana	24	8 hr
Maryland	2.7	24 hr
Massachusetts	2.7	24 hr and annual
Michigan	0.7	24 hr
	4.5	10 min (nuisance)
Minnesota	50	30 min; not to exceed more than twice per year
	30	30 min; not to exceed more than twice in any 5 consecutive days
Missouri	50	30 min; not to exceed more than twice per year
	30	30 min; not to exceed more than twice in any 5 consecutive days
Montana	50	1 hr
Nebraska	100	30 min
	10	30 day, RH >60% (welfare)
	5	30 day; RH >60% (welfare)
Nevada	80	1 hr
New Hampshire	30	24 hr
	334	Ceiling
New Mexico	100	1 hr
New York	10	1 hr
	0.7	Annual
North Carolina	1500	15 min
North Dakota	200	1 hr; not to exceed once/month
	100	24 hr; not to exceed once/yr
	20	90 day (welfare)
Oklahoma	100	30 min
Pennsylvania	5	24 hr (welfare)
Rhode Island	100	Ceiling
South Carolina	100	24 hr
Tennessee	20,000	12 hr
Texas	80	(Health; welfare)
	0.64	24 hr
Vermont	20	24 hr
Washington	0.64	
Wyoming	20	24 hr
	50	30 min; not to be exceeded twice/yr
	30	30 min; not to exceed twice in 5 consecutive days

The California State Department of Public Health (1999). states: "At the current California Ambient Air Quality Standard of 0.03 ppm, the level would be detectable by 83% of the population and would be discomforting to 40% of the population. These estimates have been substantiated by odor complaints and reports of nausea and headache (Reynolds and Kamper, 1985) at 0.03 ppm H_2S exposures from geyser emissions. The World Health Organization (WHO) reports that in order to avoid substantial complaints about odour annoyance among the exposed population, H_2S concentrations should not be allowed to exceed 0.005 ppm (7 $\mu g/m^3$) with a 30-minute averaging time (WHO, 1981; National Research Council, 1979; Lindvall, 1970)."

The U.S. E.P.A. (2000) adopted a 1-hour guideline of 30 ppb H_2S based on persistent odors, eye and throat irritation, headache and nausea reported by 6 workers exposed to a mean concentration of 0.09 ppm H_2S for approximately 5 hours in a monitoring van downwind from an oil refinery. This value was not adjusted for longer exposure conditions on the basis that 'mild irritant effects generally do not vary greatly over time', even though headaches, a serious neurological effect (Chou and Williams-Johnson, 1998), are a sign of narcosis.

7.0 RESEARCH GAPS IDENTIFIED

Several organizations have recently identified a number of research gaps. These, as well as those that are identified in this review, are summarized in Tables 33, 34, and 35. Although advances have been made in the areas of H_2S neurotoxicology, endocrinology, and cardiovascular effects, a number of knowledge gaps remain in the areas of reproductive effects, growth and development effects, and odour effects.

7.1 AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ATSDR) H_2S TOXICOLOGICAL PROFILE

ATSDR has identified the areas where scientific studies exist. Additional studies published after the ATSDR report have been identified and noted in Table 32. The ASTDR tabulated only existing studies and cautioned that this tabulation provides a reasonable picture of the focus of studies to date but does not indicate the quality of the information for any particular category. Likewise, ATSDR states the absence of studies should not necessarily be considered a data gap. A data need is substance-specific information necessary to conduct public health assessments. A data gap is substance-specific information that is missing in the scientific literature. ATSDR (1999a) notes that additional studies are required in several areas (Table 32).

Table 32 Existing Information on the Health Effects of H_2S

Endpoint	Inhalation		Oral		Dermal	
	Human	Animal	Human	Animal	Human	Animal
Death	☼	☼	◊			☼
Acute Systemic	☼	☼			☼	☼
Intermediate Systemic		☼		☼		
Chronic Systemic	☼	◊				
Immunological/Lymphoreticular	☼	☼				
Neurological	☼	◊				☼
Reproductive	◊	☼				
Developmental		☼				
Genotoxic						
Cancer	☼	◊				

☼ Existing Studies ◊ New Studies (published since ATSDR, 2000)

Table 33 H_2S Research Needs Identified by ATSDR (1999a)

Study Category	Focus Areas
Acute Duration Exposure	Studies on asthmatic subjects are needed to confirm if they are a sensitive sub-population. Studies are needed to further evaluate the effects on the eye.
Intermediate Duration Exposure	Epidemiological studies, particularly prospective and case-control studies, of populations exposed to various levels (where other pollutants are monitored and ideally, do not vary) are needed.
Chronic Duration Exposure	Studies of the carcinogenic potential in humans and animals Epidemiological studies of populations environmentally exposed due to proximity to pulp mills, sour gas plants, and geothermal energy sources are needed but only if they are accompanied by adequate exposure measurements.
Genotoxicity	Studies using H_2S in gas state are needed.
Reproductive Toxicity	Well-designed case-control studies are needed to evaluate spontaneous abortion, and epidemiological studies of other reproductive effects.
Developmental Toxicity	No studies were found evaluating developmental effects in humans. There needs to be evaluation of the developmental neurotoxicity.
Immunotoxicity	Additional studies of immune function in animals exposed by inhalation. A bacterial or viral challenge study would be especially helpful to determine whether exposure increases susceptibility to infection.
Neurotoxicity	The transplacental neurological effects are unknown. Insufficient data are available to determine whether effects are exposure route specific. Chronic effect studies after inhalation exposure are needed to determine the effects that might be seen in exposed humans.
Epidemiological and Human Dosimetry Studies	Studies examining the potential effects of chronic inhalation exposure to various H_2S concentrations are needed.
Biomarkers of Exposure and Effect	Further study is needed to corroborate airborne exposure concentrations with blood sulfide and thiosulfate levels; additional markers are needed. Additional studies of endogenous production and detoxification and distribution are needed to identify variations in susceptibility. Studies are needed to correlate neurological indices with blood sulfide concentrations and urinary thiosulfate levels.
Adsorption, Distribution, Metabolism and Excretion	There are no studies that have tracked the quantitative absorption or endogenous production of H_2S nor quantified the differences in its distribution in the various tissues to follow absorption of an external dose. Qualitative data are well known but quantitative data are lacking
Comparative Toxicokinetics	PBPK models have not been developed.
Methods for Reducing Toxic Effects	No method known of reducing absorption or the body burden. There is a need to develop an antidote, especially for treating knockdowns.
Children's Susceptibility	No data to determine the long-term effects of acute exposures to children and little information on infants, or *in utero* exposures, in particular, neurological effects.

7.2 GAPS IDENTIFIED BY THE ALBERTA COUNCIL ON RESEARCH IN AIR AND HEALTH (ACRAH) H_2S WORKING GROUP

Some research gaps identified by the ACRAH H_2S working group are given in Table 34.

Table 34 Research Gaps Identified by ACRAH H_2S Working Group

Category	Specific Focus Area
Chronic Effects	long term (chronic) low-level (ambient); repeated or subacute exposure
	long term effects of 'knockdowns"
	cumulative effects of H_2S in combination with other gases, vapors or particulates
	effects of H_2S on various sub-populations (hypersensitive, developing, young, elderly, subsequent generations)
Acute Effects	odour-induced toxicity (thresholds, paralysis, neural, behavioral, respiratory, cardiovascular responses)
Other	assessment of real-time, ambient urban/rural, point source and dispersion models as monitoring techniques
	determination of a 'no observable adverse effect level' (NOAEL) and 'lowest observable adverse effect level' (LOAEL)
	effects of stress, humidity[35], and temperature

7.3 GAPS IDENTIFIED IN THIS REPORT

This report has outlined some additional research gaps that were not previously identified by the aforementioned groups (Table 35). Further discussion of these research gaps is presented at the end of each of organ system section (above). As understanding exposure-response relationships is of primary importance, priority should be given to establish dose-response curves in all areas of investigation given in Table 35.

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[35] Early studies suggest that humidity affects the toxicity of H_2S (Segal and Suzman, 1936; DiBella, 1937).

Table 35 H_2S Research Gaps Identified in this Report

Organ System or Health Endpoint	Focus Areas
Carcinogenicity	Assess the carcinogenic potential of H_2S alone and in combination with other agents such as sulfite, sulfuric acid mists, and particulate matter containing metals such as Cu, Zn, Fe, Mg, Co. Include long-term studies in several animal species, in addition to rat and *in vitro* studies. Examine nasal, upper respiratory tract and lymphoid systems cancer incidence and prevalence among diverse H_2S-exposed groups. Investigate possible role as a co-carcinogen via non-genotoxic mechanisms such as interference with apoptosis; production of oxidant stress; and inhibition of anti-oxidant mechanisms in tissues of upper respiratory tract, lung, brain, skin, lymphatic tissue and gastrointestinal tract.
Cardiovascular properties	Investigate the role of H_2S as a risk factor for cardiovascular disease, focusing on its effects on homocysteine metabolism. Identify the specific isoenzymes associated with tissue damage found in plasma following exposure to H_2S. Establish the concentration thresholds for cardiac enzyme release. Assess the individual and combination effects of H_2S, other reduced sulphur gases on cytokine (e.g. IL-1) production and mitochondrial function in cardiac tissue.
Endocrine, Growth and Reproduction	Assess effects of H_2S on smooth muscle function in circulation, gastro-intestinal tract, respiratory tract, and biliary tract. Determine the effects of H_2S on hormone cascades involved in thermoregulation and general stress responses. Effects of H_2S on thyroid function and role in prevalence of fatigue. Investigate role of H_2S in endocrine dysfunction such as diabetes. Investigate its role as an endocrine disruptor, specifically in reproductive function, metabolic and thermoregulatory control, and carbohydrate and lipid metabolism. Address effects on growth *in utero* and after birth.
Gastrointestinal Effects	Evaluate the effects of H_2S on gastrointestinal function. Assess the combined effects of inhalation of reduced sulfur compounds and H_2S in combination with ingestion of sulfur additives in food.
Growth and Development	Conduct controlled experiments to determine the effects of H_2S exposure on reduced weight gain of farm animals. Examine the effects of H_2S on growth hormone and related hormones. Assess the role of H_2S exposure on bone, skin and connective tissue development (*in utero*). Investigate the incidence and prevalence of connective tissue diseases including arthritis among diverse H_2S-exposed groups.
Hematology	Further studies on the effects on the hematological/hemostasis systems. Address the combined effects of H_2S and methyl mercaptan in iron release from ferritin not only in the blood-forming system but also within the nervous and cardiovascular systems. Evaluate the effects of H_2S and methyl mercaptan on proteins and enzymes

Organ System or Health Endpoint	**Focus Areas**
	containing heme, iron-sulfur clusters, ferritin and possible associated secondary effects such as reactive oxygen, nitrogen or sulfur species generation.
Hepatic System	Identify the specific isoenzymes associated with tissue damage found in plasma following exposure to H_2S. Establish whether there is a combined effect of H_2S and mercaptans on liver function.
Immunity	Investigate the effects of H_2S on placental alkaline phosphatase and transmission of antibodies to the fetus. Address maternal and fetal humoral and cellular immunity interactions. Investigate interactions between immune and neural responses affected by H_2S.
Effects on Mucosal Tissues	Assess eye symptom and disease prevalence, visual acuity, and other ocular diseases in people living in areas of reduced sulphur sources e.g. pulp & paper mills, large-scale farm operations. Investigate cellular integrity alterations in mucosal and connective tissues such as upper respiratory tract, olfactory, ocular, and nervous system tissue.
Nervous System	Compare measured concentrations of H_2S exposure with neurological symptoms. Investigate brain biochemistry, physiology and function, neural growth and development assessing: i) long-term exposure effects, ii) neuromodulator role, iii) combined effects of nervous system and mitochondrial toxins; iv) cytokines affecting mitochondrial function in nervous system tissue; v) neural growth in utero; and vi) stress responses induced by H_2S and methyl mercaptan.
Odour	Examine guidelines in relation to odour responses to reduced sulfur compounds including frequency of odor occurrences and symptom responses.
Renal Effects	Establish the concentration thresholds for proteinuria and hematuria.
Reproductive Effects	Clarify the effects of H_2S alone and in combination with other gases and vapours on reproductive parameters including: birthweight, fertility, sperm quality and function.
Respiratory effects	Address interactions and long-term effects of mixtures of airborne pollutants, particularly H_2S and methyl mercaptan. Establish the concentration thresholds for edema.
Toxicokinetics: absorption, distribution, metabolism and elimination	Examine exposure routes such as olfactory nerves, the lung nerve network, and corneal epithelium. Characterize the effects of H_2S on biochemistry at the cellular and tissue level, including the interaction with iron-sulphur proteins, dietary influences, particularly with dietary sulfur, vitamins (B6, B12, niacin) and trace elements (Cu, Co, Mo, Mn, Mg, Zn, Fe). Characterize the tissue distribution and expression of metalloenzymes. Develop methods to assess effects of H_2S on overall cellular metabolic status.

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APPENDIX I

Table A1 Cancer Risks Identified in Human and Animal Studies

Reference / Study Design	Key Findings
Jappinen et al. (1989) The cancer incidence of 1,223 sawmill workers with continuous employment of at least one year between 1 January 1945 and 31 December 1961 was followed until 31 December 1980. Separate analyses were made for the 801 workers hired after 1 January 1945, and smoking habits were surveyed.	Among the men, 90 cases of primary cancer were detected versus 83.5 expected and among the women 55 cancer cases versus 44.5 expected. Skin cancer was in excess among the men, especially among those employed after 1 January 1945. Lip, mouth, and pharynx cancer and lymphomas were also slightly in excess among the men, as was leukemia among both sexes. Workplace exposure, especially to chlorophenols, may be associated with the excess skin cancer and the slight excess of lymphomas, but this finding should be further evaluated with special emphasis on well-defined exposure data.
Solet et al. (1989) Cause of death in 201 men employed in pulp and paper production plants that died between 1970 and 1984 was studied.	Significantly increased risk of malignant neoplasms (PMR 131; 95%CI 101- 166) and lung cancer (PMR 151; 95%CI 102 - 215); elevated risks were also found for lymphopoietic system cancer (PMR 190; 95%CI 82 - 375), lymphosarcoma (PMR 441; 95% CI 88 - 1292), leukemia (PMR 234; 90%CI 59 - 597) and cancer of the large intestine (PMR 147; 95%CI 59 - 303).
Neutel (1989) A mortality study of all 33,000 commercial fishermen in the Canadian Maritime Provinces that were registered with the Department of Fisheries and Oceans between 1975 and 1983, identified 1289 male deaths. The death rates were compared to Canada 1980 mortality rates.	A very high rate mortality rate, 8 times the expected rate, from water transport accidents and drowning was found. 32% of those accidents were classed as accidental drowning and submersion not otherwise classified; excluding water transport accidents. Increased risks of pancreatic cancer (SMR 2.50), and nervous system, eye, and endocrine gland cancers (SMR 2.29) were found.
Schwartz (1988) Causes of death in pulp and paper mill workers were studied.	No significantly elevated PMRs for lymphopoietic system cancers, especially leukemia were found.
Cimino and Mamtani (1987) A study of 2631 New York city sanitation workers was conducted to determine the incidence of occupational injuries and morbidity between 1973 and 1983.	The PMRs were increased for diseases of the heart (104), pancreatic cancer (196), lung cancer (109) and accidents (119), and decreased for cerebrovascular (77).

Reference / Study Design	Key Findings
Derzhko and Barilyak (1986) Rats were exposed to H_2S for 4 hours (Concentration not specified) on day 10 - 12 of pregnancy. Before H_2S exposure, rats were given 20 mg phenobarbital/kg or (glutamate, zinc sulfate, copper sulphate, iron lactate and ascorbic acid) at 50, 1, 0.5, 10, and 50mg/kg respectively.	No effects were found on the rats' bone marrow chromosomes following H_2S exposure. The number of chromosomes damaged in embryos was increased approximately 5-fold. Pre-treatment with phenobarbital increased the chromosome damage in the bone marrow cells. Administration of the glutamate and other substances prevented the chromosomal damage by H_2S
Svirchev et al. (1986) A population-based analysis of death certificates was carried out in British Columbia.	Significantly elevated proportionate mortality ratios for lymphosarcoma and reticulosarcoma for pulp mill workers (PMR 384) and sawmill workers (PMR 275) were found.
Robinson et al. (1986) The cause of death of a cohort or 3572 workers employed for at least 1 year between 1945 and 1955 in sulfate pulp and paper mills followed through to Mar 1977 was compared with US mortality rates.	No significantly elevated standard mortality ratios (SMRs) for lymphosarcoma and reticulosarcoma (SMR 207), especially in those whose latency and job tenure exceeded 20 years (SMR 364) were found.
Thomas et al. (1985) Death rates among active and retired corn wet-milling workers were identified from trade union records and compared to US males adjusting for age, race and calendar year of death.	Mortality from bladder cancer was elevated in whites (PMR 2.00) but not blacks. PMRs for leukemia (2.21) and other non-Hodgkin's lymphopoietic neoplasms (3.10) were elevated among whites. Among actively employed and retired workers, elevated frequencies of mortality from pancreatic cancer (PMR 2.16; 2.11), lymphosarcoma (4.81), diabetes mellitus (1.64), and chronic nephritis (1.64). For workers employed < 20 years, increased deaths were found for lymphosarcoma (6.76) and diabetes mellitus (1.41). For workers employed > 20 years, increased deaths were found for pancreatic cancer (2.30) bladder cancer (1.62), leukemia (1.97) and chronic nephritis (1.86). There was an elevated frequency of deaths due to diabetes (PMR 1.64). A three- fold excess of pancreatic cancer was also found among the black workers. The work history review indicated that the pancreatic cancer deaths were among those in the production process, which converts cornstarch to syrup and dextrins using hydrochloric acid, soda ash, lime and enzymes.
Mills et al. (1984) A hospital-based case-control study that reviewed the medical records of 347 patients with histologically confirmed germ-cell tumors of the testis and 347 randomly selected controls.	A significant association between work in the crude petroleum and natural gas extraction industry and risk of testicular cancer was found (OR 2.29; 95% CI 1.03 - 5.11).

Reference / Study Design	Key Findings
Milham and Demers (1984) Cause of death in two overlapping subsamples of workers engaged in the sulfite pulping process was studied.	Significantly elevated proportionate mortality ratios for cancers of the stomach, small intestine, pancreas, lymphosarcoma and reticulosarcoma were found in sulfite plus other mill types. Significantly elevated proportionate mortality ratios were found for Hodgkin's disease at sulfate, not sulfite, plus other mill types.
Gottlieb et al. (1979) A occupation comparison of 3327 lung cancer cases in Louisiana was made with 3327sex-, race-, age-, and parish-matched controls during 1960 -1975.	An approximate two-fold excess risk of lung cancer was associated with transportation equipment manufacture (mainly shipbuilding) and the fishing industry. Smaller elevations of lung cancer risk were found among older men employed in petroleum exploration and production and among male and female residents of towns where the petroleum industry was a major employer. Acadian ancestry was also associated with a higher risk of lung cancer among older male and female residents.
Hitchcock (1979) A preliminary study of the relationship between lung cancer mortality in white males and biogenic and other sources of H_2S in seven southeastern states in the Western Hemisphere salt marsh belt was undertaken.	A statistically significant increased risk of lung cancer in counties with coastal or inland marshes, and also in counties with other sulfide sources (Texas) was found.
Voors et al. (1978) Environmental correlates of cancer (smoking, residence in urban communities and residence in wetlands) were studied using multiple regression techniques.	For men, the smoking-related cancer mortality not only showed an association with residence in wetlands but also was higher in the Louisiana wetlands that in the remainder of the US. The authors suggest that a 'wetlands-associated substance' may promote respiratory cancer.
Harrington et al. (1978) A comparison occupation statements on death certificate for 858 white males who lived in coastal Georgia counties and who died of lung cancer during 1961 and 1974 were compared with age- and place of residence-matched controls.	A two-fold excess risk of lung cancer was associated with the construction industry. No elevated risk of lung cancer was found for wood and paper workers in the urban areas of Savanna, Brunswick or Waycross. However, a three-fold increase was found for the remaining coastal counties where the wood and paper industry was the largest employer.

Table A2 *In vitro* **Studies - Mutagenicity, Chromosomal Alterations, Other**

Reference / Study Design	Key Findings
Carlsson et al. (1988) In growing cultures of *S. typhimurium* strain TA102, lethality and mutagenicity of hydrogen peroxide and prior exposure to 100 uM sodium sulfide was tested.	Exposure to sodium sulfide significantly increased the lethality and mutagenicity of hydrogen peroxide. There was a corresponding decreased in catalase activity that was believed to explain the increased mutagenicity of hydrogen peroxide. The activities of superoxide dismutase, glutathione reductase or NADPH-dependent alkyl hydroperoxide reductase in the bacterial cells were not affected by sodium sulfide.
Berglin and Carlsson (1986) The effects of L-cysteine and sodium sulfide on the mutagenicity of hydrogen peroxide were tested in a *S. typhimurium* tester strain.	Addition of L-cysteine or sodium sulfide significantly increased the mutagenicity of hydrogen peroxide. L-cysteine itself was mutagenic to S. typhimurium TA102.
Berglin et al. (1982); Berglin and Carlsson (1985) The effects of L-cysteine and sodium sulfide on the mutagenicity of hydrogen peroxide were tested in an *E. coli* tester strain.	L-cysteine potentiates by 100-fold the hydrogen peroxide induced killing of a growing culture of *E. Coli* K 12 and the killing is related to DNA breakage in the cells.
Gocke et al. (1981) Sodium sulfide (0.1 - 5 umol per plate) was tested in the Ames plate assay. 50mM Na_2S was fed in 5% saccharose to *Drosophila* in the Basc test to evaluate mutations. 0.2 - 0.8 mmol/kg sodium sulfide was tested in the micronucleus test on mouse bone marrow cells.	A weakly mutagenic response was observed. With addition of rat liver S-9 from Arochlor-pretreated rats abolished the mutagenic activity. A mild elevation in X-linked recessive mutation frequency was found in the initial brood but not in subsequent broods.
Magnusson and Ramel (1978) Thioacetamide was tested for mutagenicity in *Drosophila*.	Thioacetamide, which in aqueous solution decays to acetamide and hydrogen sulfide, was found to be mutagenic.

Table A3 Cardiovascular System Effects: Human Studies

Reference \ Study Design	Key Findings
Neutel (1989) A mortality study of all 33,000 commercial fishermen in the Canadian Maritime Provinces that were registered with the Department of Fisheries and Oceans between 1975 and 1983, identified 1289 male deaths. The death rates were compared to Canada 1980 mortality rates.	A very high rate mortality rate, 8 times the expected rate, from water transport accidents and drowning was found. 32% of those accidents were classed as accidental drowning and submersion not otherwise classified; excluding water transport accidents. In the < 44 year age group, increased mortality rates, compared to the rest of Canada were found for all heart disease (SMR 1.34) and ischemic heart disease (SMR 1.36).
Jappinen (1987) A retrospective cohort study of 3520 Finnish pulp and paper workers employed continuously for at least one year between 1945 and 1961 were studied. The cohort was subdivided into subcohorts of sulfite, sulfate, paper mill, board mill, maintenance and power plant workers.	Overall mortality for the entire cohort did not differ from that expected however death from diseases of the circulatory system among the men was increased (SMR 121; 95% CI 109 - 134) due to an excess of coronary deaths (SMR 128; 95%CI 114 - 144). The increase in coronary deaths were among workers in the sulfite mill, sulphate mill, paper mill, maintenance department and power plant and could not be attributed to differences in smoking habits. Increased mortality due to ischemic heart disease among sulfate workers (SMR 142; 95% CI 115 - 174) and power plant workers (SMR 158; 95% CI 122 - 200) was found. Sulfite workers (SMR 131; 95% CI 97- 173) and paper mill workers (SMR 138; 95% CI 95 - 193) also showed increased risks.
Cimino and Mamtani (1987) A study of 2631 New York city sanitation workers was conducted to determine the incidence of occupational injuries and morbidity between 1973 and 1983.	The PMRs were increased for diseases of the heart (104), pancreatic cancer (196), lung cancer (109) and accidents (119), and decreased for cerebrovascular (77).
Hoidal et al. (1986) Case report of two workers exposed to roofing asphalt fumes inside a tank. The re-created gas concentrations were H_2S 650 - 700 ppm; carbon monoxide 4000 ppm; propane 4800 ppm; methane 18540 ppm; aliphatic hydrocarbons 8300 ppm; oxygen 10 - 12%.	A 35 year-old man was found in asystolic cardiopulmonary arrest after an estimated 20 min. of exposure. Ventricular fibrillation was observed and following defibrillation, a normal sinus rhythm was established. After transport to the hospital, ECG showed sinus tachycardia. The second worker, after about 10 minutes of exposure, was initially comatose and apneic. He regained spontaneous respirations and became combative; generalized seizure activity was observed during transport and at the hospital; sinus tachycardia was observed. Carbon monoxide poisoning was ruled out by the low values of carboxyhemoglobin levels obtained shortly after rescue.
Arnold et al. (1985) Medical records of H_2S exposed workers who submitted compensation claims were reviewed.	Of 250 claims reviewed, 11 ECGs were done. Four of those 11 showed abnormalities including two cases of sinus bradycardia, one of ST-T changes with flutter fibrillation and one with changes consistent with hyperkalemia.

Audeau et al. (1985) Case reports of 4 workers exposed to H_2S in a pelt room when sulphuric acid was added to a pickling drum.	Blood pressure increases were noted in 3/4 workers exposed to H_2S in a pelt room, which returned to normal a few hours or a few days later. The electrocardiograms were normal.
Milham and Demers (1984) Death records of 2113 United States and Canadian members of the Pulp, Sulfite and Paper Workers Union were examined and compared to the general population.	An excess of deaths due to circulatory diseases (proportionate mortality ratio [PMR] of 114) was reported. The PMR increased slightly to 121 when sulfate plus other workers were considered.
Donham et al. (1982) Case reports of 6 workers exposed to gases from liquid manure storage pits.	One out of six workers exposed were reported to have tachycardia upon admission to the hospital.
Ravizza et al. (1982) Description of exposure to H_2S.	No change in blood pressure was noted despite presence of cardiac irregularities.
Peters (1981) Case report of an H_2S poisoning in a hospital.	Sinus tachycardia was found in men who completely recovered after exposure to H_2S.
Stine et al (1976) A worker was exposed to H_2S generated from a sodium sulfide waste solution dumped onto acid waste material.	Supraventricular tachycardia and left bundle block was noted. The effects were temporary.
Shults et al. (1970) Case report of acute methyl mercaptan inhalation poisoning.	Signs of tachycardia, elevated blood pressure and severe anemia developed within 24 hours, followed by death.
Thoman (1969) A woman who attempted to clean a well with muriatic acid was exposed to an unknown concentration of H_2S.	Extreme tachycardia and hypotension was observed in an exposed woman and hypertension was observed in an exposed man.
Kemper (1966) Case report of a man who had severe H_2S poisoning with collapse and respiratory failure.	ECG evidence of myocardial ischemia was noted during the early phase of acute illness but gradually disappeared over a period of 15 days.
Krekel (1964) Workers were exposed for less than 5 minutes after a spill of sodium sulfide that released H_2S.	Electrocardiograms taken on two workers about 2.5 hours after an acute H_2S exposure showed cardiac arrhythmias. One individual had a negative P wave and the other had a continuous arrhythmia due to atrial flutter, which for both returned to normal within 24 hours.
Kaipainen (1954) A 48-year old farmer collapsed while shoveling manure.	He continued to have convulsive seizures after resuscitation. The ECG changes suggested myocardial infarction. The patient recovered but a slight persistent dizziness remained.

Hurwitz and Taylor (1954) Case report of a 46-year old sewer worker who was overcome in a manhole for 30 minutes. He was cyanotic, suffered generalized spasms and required artificial respiration.	A week later, he could move and speak only with great effort. One month later, he still exhibited neurological deficits. The ECG showed evidence of small anterolateral infarct with a right bundle block. Three months later, although ambulatory, the patient still suffered from anginal pain upon exertion. It is noted that 'cardiovascular degeneration has been described as an occupational hazard in sewer-gas workers', citing a 1953 paper.
Helfors (1938) Case report of a man inhaling H_2S.	The man became dizzy, suffered chest constriction, irritation of throat, diffuse bronchitis, diarrhea and vomiting. The next day there was sugar in the urine and blood sugar was 126 mg%. The man died soon of angina pectoris.
Hertz (1932) Case report of acute poisoning of a 27- year old worker in a chemical factory.	The illness turned into a chronic heart ailment.
Oliver (1911) The working conditions and occupational hazards of Sicilian sulfur miners were reviewed. The health risks posed by constant exposure to sulfur vapors, sulfurous acid and hydrogen sulfide are noted.	Many of the young boys working in the mines have stunted growth. A high mortality due to heart and kidney disease among the miners was discussed.

Table A4 Cardiovascular System Effects: Animal Studies

Study Design	Key Findings
CIIT (Chemical Industry Institute of Toxicology) (1983a,b,c) Fisher-344 or Sprague-Dawley rats or B6C3F1 mice were exposed in an inhalation chamber to 10.1, 30.5, or 80 ppm H_2S for 6 hr/d, 5 days/wk for 90 days.	No treatment-related histopathological effects were noted on the cardiovascular system.
Savolainen (1982) H_2S water solution (265 mg/L) was injected into the ear of a rabbit.	Prolongation of the diastole, lowered heart rate and changes in the T-wave were observed. Decreased heart frequency has been reported in other mammals given intravenous injections of 0.5 - 10 mg/L H_2S.
Dwornicki (1979) Rabbits were exposed to 72 ppm 1 hr/day for 14 days.	Various arrhythmias, including ventricular extrasystoles were observed after several days of exposure. In the myocardium, decreased staining of alkaline phosphatase, slightly reduced succinic dehydrogenase and unaltered ATPase was found.
Higuchi and Fukamachi (1977) Male Wistar rats were exposed to 100 - 200 ppm H_2S for 1 hour.	A temporary yet marked increase in blood pressure was observed.
Aitbaev et al. (1976) [cited in Toxcon, 1987] Albino rats were exposed to 7 ppm H_2S for 6 hr/day for 4 months.	Compared to controls, a 16.7% decrease in succinate dehydrogenase activity in heart was found.
Elebekova et al. (1976) Rats were exposed to 3.5 or 6.7 ppm H_2S for 6 hr/day for 4 months.	Reduced levels of liver glycogen (68 and 91.9%), lower blood sugar (50.2, 69.1%), increased blood lipids (98.6, 202.6%) and lower liver lipids (38.8, 27.9%) were found.
Kuwai (1960) Rabbits were exposed to 20 - 25 ppm H_2S for 4 hr/d for 150 days in an inhalation chamber	An initial increase in free cholesterol and a fall in the ester ratio that returned to normal levels after a short period were observed.
Kosminder et al. (1967) Rabbits were exposed to 71 ppm H_2S for 1.5 hours and then 0.5hr/d for 5 days. Histochemical staining of the myocardium was also done.	Ventricular polarization occurred after 1.5 hr exposure. Arrhythmias that persisted several days post-exposure were observed after the 5-day exposure. A reduction in ATP-phosphohydrolase and NADPH oxido-reductase was found in myocardial cells by histochemistry.

Pavlenko (1940) Various doses of H_2S (300 mg/L of saline solution) were injected into the blood stream of rabbits.	H_2S caused a brief lowering of blood pressure previously raised by adrenalin, which later returns to a high level. H_2S prolongs the pressor action of adrenalin. Small doses (1 mL) of H_2S raise the reactivity of the animal to subsequent doses of KCl. H_2S increases the vagus sensitivity to electrical excitation. Preliminary treatment with H_2S of sensitized animals intensifies the anaphylactic shock and the effect is lethal in more cases than in controls. The reaction of vegetative centers to H_2S varies according to their initial physiological state.
Sorokin & Ol'shanetskaya (1941) Rabbits were intravenously injected with an artificial mineral water containing 180 mg/L free H_2S at the rate of 1 mL/sec.	IV infusion of artificial mineral water decreased the heart rate when the total amount introduced into the blood was 1.2 - 2.5 mL, equivalent to 0.25 - 0.5 mg free H_2S. When 3, 5, and 7 mL was injected, the rate of contractions fell from a normal of 250 - 300/min to 80 - 100/min and remained at this level for 1 - 3 min after injections were discontinued. The effect was entirely due to prolonged diastole. Injection of 5 mL produced extrasystole in the auricle as well as in the ventricle. At times, hyposystole and asystoles of ventricles were observed.

Table A5 Endocrine System Effects - Human Studies

Reference / Study Design	Key Findings
Henneberger et al. (1989) Cause specific mortality was determined for 883 white male paper company workers in Berlin, New Hampshire. Different exposure groups were created on the basis of having worked in the pulp mill, the paper mill, or elsewhere in the paper company. A standardized mortality ratio (SMR) analysis was used to compare death rates for each of the exposure groups with United States national rates.	Among pulp mill workers, the number of cancers of the digestive system was raised and the SMR for pancreatic cancer was especially high (SMR = 305, 95% CI 98 - 712). An increased mortality from diabetes mellitus (SMR 285; 95% CI 104 - 620) was found.
Schwartz (1988) A proportionate mortality ratio (PMR) analysis of 1071 deaths in pulp and paper mill workers in New Hampshire was done during 1975-85.	An increased risk for diabetes mellitus (PMR 146; 90% CI 103 - 207) among pulp and paper workers from New Hampshire was found.
Thomas et al. (1985) Death rates among active and retired corn wet-milling workers were identified from trade union records and compared to US males adjusting for age, race and calendar year of death.	There was an elevated frequency of deaths due to diabetes. A three- fold excess of pancreatic cancer was also found among the black workers. The work history review indicated that the pancreatic cancer deaths were among those in the production process that converts cornstarch to syrup and dextrins using hydrochloric acid, soda ash, lime and enzymes.
Bulatova et al. (1968) [cited in AB Health, 1988) 2465 high-sulphur petroleum refinery workers in two cities exposed to H_2S at unspecified concentrations were compared with 601 machine-tool and 706 railway workers.	The incidence of cholecystitis (gall bladder disease), cholangitis (bile duct disease), and cholelithiasis (gallstones) and the number of workdays lost was greater in the oil refinery workers. The morbidity of oil refinery workers with over 5 years service was greater than that of those with less time in service. Contrast cholecystography showed that 56 of 4 74 cases of biliary dyskinesia in the oil-refining workers were of a hypermotor type and 12 were of a hypertonic type.
Helfors (1938) Case report of a man inhaling H_2S.	The man became dizzy, suffered chest constriction, irritation of throat, diffuse bronchitis, diarrhea and vomiting. The next day there was sugar in the urine and blood sugar was 126 mg%. The man died soon of angina pectoris.
Daniel & Popesco-Buzeu (1931) The action of sulfur water on dogs and normal and diabetic patients is contrasted with the action of distilled water.	In all cases where the sulfur mineral water was used there was a pronounced diminution of blood sugar, while distilled water had no effect.

Table A6 Endocrine System Effects - Animal Studies

Reference / Study Design	Key Findings
Hayden et al. (1989) The effects of 0.15 - 15 uM sodium sulfide on rat uterine contractile response was investigated.	Sulfide reversibly attenuated the contractile response of the uterus to oxytocin without affecting angiotensin II responsiveness.
Kruszyna et al. (1985) Guinea pig ileum, rat pulmonary artery and rabbit aorta function was evaluated by exposing tissues to relaxants and contractants (sodium nitroprusside (nitric oxide producing agent), and histamine or acetylcholine, respectively.	Sulfide augmented the relaxation induced by nitroprusside and reversed the effects of nitric oxide hemoglobin, nitroglycerin, and nitrite. Sodium sulfide also reversed the spasmolytic effects of azide and hydroxylamine.
Novikova et al. (1980) cited in Beauchamp et al. (1984) Rats were exposed to H_2S to 14 and 28 ppm H_2S for 4 hr/day, 5 days/week for 4 months.	The animals developed dose-dependent lesions of the thyroid gland. Microfollicles developed and were lined by proliferating and hypertrophied thyroid secretory cells.
Hays (1972) [cited in NRC, 1981] Mice were exposed to 10, 20, 30, 50, and 100 ppm H_2S. Goats were exposed to 50 and 100 ppm H_2S for 4 days. Lactating dairy cattle were exposed for 21 days to 20 ppm H_2S and were observed for 21 days.	Rectal temperatures were reduced in all exposures except 10 ppm, in a dose-dependent manner. Compared to the rectal temperature in controls of 38C, the rectal temperature 24-48 hours after exposure to 20 ppm was reduced to 34.6 - 34.8C. Goats exposed to 50 and 100 ppm for 4 days were generally stressed as shown by a 48 and 55% increase, respectively, in plasma cortisol level, and fever. Compared to preexposure levels (16.59 kg of milk/cow/day), milk production was reduced during exposure (15.35) and post-exposure interval (14.07 kg/cow/day).
Braginskaya et al. (1968); [cited in Gangolli, 1999) Albino mice were expose to 0.1 mg H_2S /L (70 ppm) for 6 hr/day for 9 months.	Exposed animals exhibited decreased muscular strength, static and dynamic performance, and endurance capacity, together with decreased glycogen, ATP, and creatinine phosphoric acid levels and increased lactic acid levels in the muscles and liver.
Obal & Incze (1955)	Frog muscle, intoxicated with H_2S during rhythmical activity becomes more quickly fatigued than control muscle, and its mechanical efficiency decreased 10-70%. A tetanic contraction of an intoxicated muscle lasts only a short time, only 30-70% of that of the control.
Zetler (1951)	Pre-treatment of adult white mice uterus with H_2S increased its contractions in anoxia and increased the anaerobic response to posterior pituitary extract.
Evans et al. (1942)	Cysteine inactivates pregnant-mare serum gonadotropin. H_2S does not inactivate pregnant mare serum under the conditions used. H_2S in the presence of 40% urea produces 60% inactivation.

Table A7 Gastrointestinal System Effects: Human and Animal Studies

Reference / Study Design	Key Findings
Reynolds and Kemper (1983)	Odor complaints, nausea and headaches have been reported near geysers where H_2S concentrations were measured at 30 ppb.
CIIT (1983 a,b,c) Fisher-344 or Sprague-Dawley rats or B6C3F1 mice were exposed in an inhalation chamber to 10.1, 30.5, or 80 ppm H_2S for 6 hr/d, 5 days/wk for 90 days.	No treatment-related histopathological changes were observed in the GI tract of any exposed animals.
Sandmeyer (1981)	Accidental exposure of 28 students to an approximation of 4-ppm methylmercaptan for several hours resulted in headaches and nausea, which reversed within 24 hours of removal from a contaminated area.
Beruashivili (1980) White rats ingested thermal water containing 10 - 12 mg H_2S /L (duration and amount not given).	Disturbances of digestive function were reported.
Schielerr (1973)	Increased incidence of mental depression, dizziness and blurred vision, nausea, loss of appetite were reported at 0.11 - 0.14 mg/m3 H_2S (0.08 - 0.1 ppm).
Prouza (1970) Accidental exposure of 9 workers in a viscose plant while repairing a tank heater is described. H_2S levels measured 4.3 hours after exposure were 11 - 25 ppm.	4/9 workers complained of nausea, weakness, and chest pain and were hospitalized. After 1 week no symptoms were found on internal, eye, neurological, psychiatric exam or EKG. 5/9 workers were only briefly affected. All were examined 2 years later and no after-effects were found.
Indiana Air Pollution Control Board (1964) An intermittent air pollution episode in Terre-Haute over a 2-month period was described. H_2S concentrations were measured between 0.003 - 11 mg/m^3 (2 ppb – 8 ppm)	Increase incidence of nausea, loss of sleep, shortness of breath, and headaches following chronic H_2S exposure.
Sukhanova (1962a)	The chronic action (4 months) of cracking gas containing 0.05 – 0.07 mg H_2S/L on white rats led to a lowering of uropepsin activity as detected by a modified West-Idel'son method. After stimulation with pilocarpine, the intensity of inclusion of radioactive methionine in the pancreatic protein was lower after the action of cracking gas.
Sukhanova (1962b) Rats were poisoned with 0.05 – 0.07 mg/L H_2S for 4 hr/day for 4 months.	Uropepsin activity decreased following exposure (milk coagulation time increased to 500 sec; in control rats the time was 300 sec).
Loginova (1957) [cited in Toxcon, 1987] Effects of chronic H_2S exposure (4 - 210 ppb) in a mixture with CS_2 and SO_2 are described.	Headaches, weakness, nausea, vision problems and higher general morbidity rates in those households with greater than 36 ppb H_2S.

Leopold & Treutler (1952) A clinical and x-ray study was made of 205 workers in a low-temperature carbonization plant (lignite) for maladies of the stomach, which were found in nearly 50% of the cases.	Hypochrome anemia was present only in a few of the cases. The number of erythrocytes was increased in 30 cases, while leukocytosis was present in a larger number. Blood bilirubin and Takata reaction were normal. Normal acidity of the gastric juice was found in 54 of 125 cases tested, the rest giving 8 hyperacid, 36 subacid and 27 anacid values. Only in few cases were the gastric disturbances related to subchronic acute CO-H_2S intoxication. The social conditions were believed to be the main disposition factor for gastric complaints, but possible irritation of the gastric mucosa by the producer gas should also be considered.
Ludany et al. (1952)	The presence of H_2S (10 - 20 mg%) in a loop of dog intestine caused an average 20% decrease in the amount of glucose absorbed in 20 minutes; and *in vitro* phosphorylation of glucose by the mucosa is markedly inhibited. Xylose absorption is not affected.
Ahlborg (1951) Symptom prevalence of shale oil workers was compared to controls.	72% of 459 workers usually exposed to 20 ppm or more of H_2S complained of one of more of the following symptoms: fatigue, somnolence, lack of initiative, decreased libido, loss of appetite, headache, irritability, poor memory, anxiety, dizziness, itching, eye irritation, respiratory tract irritation, gastrointestinal disorders, insomnia and backache. Acute higher exposures gave rise to more severe, often neurological problems. These included: signs of cerebral and extrapyrimidal damage, facial paralysis, prolonged reaction time, absent of abnormal reflexes at both cranial and spinal nerve levels, poor memory for recent events, depression, either timidity or fierceness, and with an epileptic-like seizure. Sequelae of acute H_2S exposure occurred even when the affected individual had not lost consciousness. In one person, gastritis persisted for 1 year after exposure at unspecified concentrations. Another worker developed problems maintaining equilibrium 6 months after acute exposure to H_2S at an unspecified concentration. The disturbance of equilibrium still persisted after 3 years.
Legge (1934) 78 workers in the spinning department and acid cellars of an artificial silk factory in the Netherlands were surveyed in 1922. H_2S concentrations were reported to vary between 20 and 35 mg/m^3 (14 and 25 ppm). No information on exposure duration was available.	Complaints of burning and smarting of the eyes was made by 25/78 workers. 32/78 workers complained of headache. > 25% experienced dizziness 21/ 78 of loss of appetite 20/ 78 of weight loss.
Kmietowicz (1931)	Large doses of H_2S introduced into the intestine first caused an acceleration of respiration and then cessation with death resulting. The blood pressure was raised and the heart rate was lowered with increased amplitude. Artificial alkalosis, acidosis and the injection of morphine increased the toxicity of H_2S, with alkalosis having the greatest effect. The absorption of H_2S is greater in the small intestine than in the large intestine.
Kranenburg and Kessener (1925) Eye injuries since 1921 caused by H_2S in beet sugar mills and rayon factories in the Netherlands were surveyed.	Eye damage was found in 95 cases where sewage polluted water had been used to wash beets. The symptoms included pain, photophobia, epiphora (increased tears), inability to open the eyes, and headaches. Levels of H_2S associated with conjunctivitis were 20 - 35 mg/m3. Chronic exposure to similar levels also caused headache, dizziness, fatigue, nausea, poor appetite, and loss of weight.

Table A8 Growth and Developmental Effects: Human and Animal Studies

Reference / Study Design	Key Findings
Saillenffait et al. (1989) Pregnant Sprague-Dawley rats were exposed to 50, 100 or 150 ppm H_2S on gestation days 6 - 20.	Exposure at 50 ppm had no effect on body weight gain or absolute weight gain (minus the gravid uterine weight). Absolute weight gains were decreased at both 100 and 150 ppm. Decreased body weight gains were observed which was statistically significant only at the higher dose.
Gagnaire et al. (1986) Sprague-Dawley rats were exposed to 50 ppm H_2S for 5 days/wk for 25 weeks.	No effects on body weight were found.
Alberta Environmental Center (1986) Rats were exposed to 40 ppm H_2S for 6 hours.	The animals lost weight, on average 19.3 g, following the 6-hour exposure to 40 ppm compared to weight loss of 9.0 g in the chamber control animals.
Kluczek & Kluczek (1985) 2wk to 3-months old calves were raised in stables with elevated air concentrations of carbon dioxide, ammonia, and H_2S (2-3 fold above control).	The growth rates of the calves were below those of control calves.
CIIT (1983) Mice were exposed to 80 ppm H_2 for 90 days, 6hr/d, and 5 d/wk in an inhalation chamber.	A depression in body weight gain was observed.
CIIT (1983) Rats were exposed to 10, 30, or 80 ppm H_2S 6hr/d, 5 d/wk for 90 days. Clinical hematologic, urinary, and serum chemistry parameters, and detailed necropsy and microscopic examinations were done. Also, detailed neurologic and ophthalmologic studies were conducted.	After the first week of exposure, significant reductions in body weight gains in all groups and both sexes were found.
Savolainen and Tenhunen (1982) Male rats were given water containing sodium sulfite (Na2SO3) over 10 wks, for a total ingested dose of 5.6, 15.5, 28.9, and 41.0 mmol/kg for 1, 3, 7, and 10 weeks respectively,	No weight gain differences for any of the interim time periods were found. At ten weeks, compared to controls, reduced cerebral RNA, reduced glutathione levels, and at 7 and 10 weeks, reduced brain and liver heme synthase levels were found.
Stolpe et al. (1976) Rats were exposed to 10 ppm H_2S for 41 days.	Significantly less weight gain was observed at the end of the exposure period compared to controls.

Aitbaev et al. (1976) Albino rats were exposed to 7 ppm H_2S for 6 hr/day for 4 months in an inhalation chamber.	Retarded body weight gains were observed.
Curtis et al. (1975) Pigs were exposed to 8.5 ppm H_2S for 17 days	No difference in body weight gain as compared to controls was found.
Hays (1972) Exposure to 20 ppm H_2S in an inhalation chamber for 48 hours. Mice were exposed to 10 ppm H_2S for 5 days continuously.	Food and water intake deceased 20%, rectal temperatures decreased 3.7C, and animals lost weight following exposure. Body temperature dropped significantly after 48 hours exposure but was not significantly different from the controls during the rest of the exposure period. Food and water intake was decreased 60% during the first 24 hours but was decreased only 28% by day 5, compared to controls.
Hays (1972) (cited in Chalmers, 1997; p 83 - 87) Three Holstein lactating cows exposed continuously to 20 ppm H_2S for 21 days and were observed for 21 days after exposure.	Milk production decreased from 16.59 to 15.35 to 14.07 kg/cow/day during the pre-exposure, exposure, and post-exposure intervals, respectively. Feed consumption was 14.17, 14.99 and 13.68 kg/cow/day for pre-exposure, and post-exposure intervals, respectively. Average heart rates were 65.6, 63.1 and 62.0 beats/min for the corresponding periods.
Hays (1972) Goats were exposed to 10, 50 and 100 ppm H_2S in an inhalation chamber continuously for 4 days.	In the 10-ppm exposure group, reduced food (20%) and water (63%) intake on the first day was observed but returned to near-normal levels thereafter. For the 50 and 100-ppm exposure groups, feed consumption decreased on day 2 and remained depressed through the remaining exposure period. A 37% decrease for the 100-ppm group was observed.
Misizkiewicz et al. (1972) Male wistar rats were exposed to a) 0.03 or b) 0.3 ppm CS_2 alone, c) a mixture of CS_2 (0.03 ppm) and H_2S (0.07 ppm), or d) a mixture of CS2 (0.3 ppm) and H2S (0.7 ppm) continuously (except for feeding) for 160 days in an inhalation chamber.	Compared to controls, the weight gains were reduced by 7.5, 15.4, 23.3 and 28.9% for the four exposure groups, respectively.
Nordstrum (1975) 36 calves were exposed to H_2S (20 or 150 ppm) in enclosed chambers for 7 days of continuous exposure.	Compared to controls, feed consumption was decreased by 3.5% and 26% for 20 and 150-ppm exposures, respectively. Water intake was reduced at the higher exposure. Appetite tended to recover during the latter part of the exposure interval.

Sinitsyn (1962) Rats in 4 groups were exposed to H_2S (0.58 - 13.2 mL/L air). The age groups were 1-8 days, 16-28 days, 3 months, and 6 months and older.	Observed toxicity was 10 - 12 times higher in the oldest than in the youngest, about equal in the two oldest age groups, and 15 - 20% higher in the 16-28 day age group than in those 6 months or older.
Isobe (1933)	Young white or wild rats were more resistant than adults to CO, HCN, H_2S, phenylhydrazine and aniline. Removal of the spleen did not influence susceptibility.
Krasovitskaya et al. (1970) The health of children who lived in a polluted area around an organic synthesis plant was evaluated. H_2S, CS_2, SO_2, SO_3, N oxides, phenol and dust were present in zones 500 - 10,000 m from the plant. The greatest contamination was with H_2S, N oxides and dust.	The maximum allowable concentrations were exceeded in 34% of the analyses. Children living in the polluted area showed a lag in physical development, an elevation in the percentage with anemia and chronic rhinitis, and more frequent disturbances in central nervous system functions.
Sandage (1961) [cited in Toxcon, 1987] Mice were exposed to 20 ppm H_2S for 90 days continuously.	A statistically significant weight loss after 90 days, and decrease in swimming endurance compared to controls was found.
Fyn-Zhuy (1959) Male rats were exposed to 0.2 or 10mg/m3 H_2S 12 hrs/day for 3 months.	A lag in weight gain occurred in some of the high dose rats.
Glebova (1960) [cited in Illinois Institute for Environmental Quality, 1974]	Small concentrations of H_2S (0.1 - 10 mg/m3) (70 ppb - 7 ppm) have produced a symptom complex usually manifested as undernourishment, delayed growth, general weakness, and retarded physical and neurophysical development (e.g. Began walking late and teeth were slow in cutting through), as well as assorted gastro-intestinal disturbances.
Duan (1950) Albino rats were exposed to 10 ppm H_2S for 12 hr/d for 17 weeks in an inhalation chamber.	Weight gains were retarded relative to controls, whereas no difference in weight gain was observed in animals exposed to 0.01 ppm H_2S.
Oliver (1911) The working conditions and occupational hazards of Sicilian sulfur miners were reviewed. The health risks posed by constant exposure to sulfur vapors, sulfurous acid and H_2S are noted.	Many of the young boys working in the mines have stunted growth. A high mortality due to heart and kidney disease among the miners was discussed.

Table A9 Hematological System Effects: Human Studies

Reference / Study Design	Key Findings
Klingberg et al. (1988) The differences in heme parameters were measured between 18 pulp and paper maintenance workers and controls not exposed to sulfides matched for age, smoking and physical activity. Heme synthase (HS), delta-aminolevulinic acid synthase (D-ALA-S), iron, transferrin, and ferritin levels were measured in the blood.	Exposure to sulphur compounds was estimated from registered peak levels and measured mean levels. The mean concentrations of the sulfides were low, generally below the detection limits (< 0.2 ppm for methylmercaptan and < 0.05 ppm for dimethylsulfide and dimethyldisulfide). H_2S was not measured. D-ALA synthase and HS activities were lower, although not statistically significant, among the exposed subjects than the referents. The concentrations of serum iron and transferrin were elevated and the ferritin was low among the highly exposed subjects. This combination of findings is not known to occur spontaneously or as a result of known disease which suggested to the authors that low level sulfide exposure disturbs heme synthesis and alters iron uptake.
Audeau et al. (1985) Case report of 4 cases of workers exposed to H_2S in a pelt room.	Complete blood counts were normal for 4 individuals overcome by unknown H_2S concentrations in a pelt room.
Tenhunen et al. (1983) 17 exposed pulp production workers (H_2S 8 hr time weighted average of 0.05 to 5.2 ppm), methylmercaptan (0.07 to 2.0 ppm) and dimethylsulfide (0.03 - 3.2 ppm)	Reduced activities of D-ALA synthase, ALA-dehydrase, and ferrochelatase relative to reference ranges of 8, 2, and 5 workers respectively were found. Also, compared to control values, low erythrocyte protoporphyrin levels were found in 7 workers. Low D-ALA synthase and heme synthase activities were found in one patient with H_2S intoxication 1 week after the event and the enzyme activities returned to control levels 2 months later even though the erythrocyte protoporphyrin remained abnormally low. None of the workers had clinical anemia, however the bone marrow yield was 'scanty' suggesting a reduced compensation capacity. In vitro, H_2S inhibited D-ALA synthase activity 85% at 10 mmol/L concentration. Thiosulfate anion inhibited D-ALA synthase 18% and heme synthase 43% at 10 mmol/L.
Peters (1981) Case report of a worker exposed to emissions from a plugged drain trap treated with sulphuric acid.	On admission to hospital, leukocyte counts were elevated to 13,700/mm3, however the differential was normal. Sulfhemoglobin was increased to 7.9% of total blood volume (normal $< 0.5\%$).

Morse and Woodbury (1981) Case report of a 16-year old farm worker overcome by emissions from an underground liquid manure storage tank.	Focal hemorrhages in the hilar portion of each lung and small petechial brain hemorrhages were found.
Biesold et al. (1977) An electron microscopic examination of lung tissue from a 7-year old boy that died after being exposed to H_2S from a latrine.	A severe alveolar and interstitial edema of the hemorrhagic type was found. Evidence of direct toxic effect on the endothelial and epithelial barrier of the alveoli was observed. Widespread damage to the squamous epithelium, partially denuding the basement membrane, and endothelial gaps were found often covered with microthrombi.
Stine et al. (1976)	Bilateral subconjunctival hemorrhages was one of the physical signs found 5 hours after an incident of acute exposure.
Shults et al. (1970) A laborer was handling tanks of methyl mercaptan was hospitalized in a coma.	Acute hemolytic anemia and methemoglobinemia was reported. This individual was later found to have glucose-6-phosphate dehydrogenase deficiency.
Krasovitskaya et al. (1970) The health of children who lived in a polluted area around an organic synthesis plant was evaluated. H_2S , CS_2, SO_2, SO_3, N oxides, phenol and dust were present in zones 500 - 10,000 m from the plant. The greatest contamination was with H_2S, N oxides and dust.	The maximum allowable concentrations were exceeded in 34% of the analyses. Children living in the polluted area showed a lag in physical development, an elevation in the percentage with anemia and chronic rhinitis, and more frequent disturbances in CNS functions.
Benini & Colamussi (1969) [cited in Toxcon, 1987] Exposures to 0 - 7 ppm H_2S for 3 days are described.	Occasional slight and irregular changes in serum iron, transferrin and urinary sulfates fractions are reported.
Ahlborg (1951) Workers in the shale industry were sometimes exposed to > 20 ppm H_2S.	No changes in hematological parameters were found.□
Slovtzov (1917)	Long-term respiration by air containing H_2S can evoke a temporary anemia, which is followed by an increased quantity of red corpuscles. The body weight is decreased. H_2S introduced into the blood causes swelling of the red blood cells and the blood loses its capacity to unite with CO_2 and to absorb oxygen.

Table A10 Hematological System Effects: Animal Studies

Reference / Study Design	Key Findings
Baskurt (1988) Rats were exposed to 0.87 ppm SO_2 for 24 hrs.	Compared to controls, exposed rats had increased hematocrit, sulfhemoglobin, and erythrocyte osmotic hemolysis. The whole blood and packed cell viscosities were lower than controls.
Tenhunen and Savolainen (1987) Rats were injected intraperitoneally with Na_2S (150 umol/kg body weight) and then, 25 hours after, treated with heme to evaluate the effects on blood delta-amino levulinic acid synthase and heme synthase activities.	The sulfide dosing decreased the activity of both enzymes. The heme treatment over-corrected the sulfide-induced inhibition of heme synthase.
Savolainen et al. (1985) Rats injected intraperitoneally with 0.15 mmol/kg body weight sodium sulfide.	Reduced reticulocyte D-ALA-S and heme synthase activities 24 hours after injection were found. Liver D-ALA-S and heme oxygenase activities were also reduced. These effects were corrected with injection of heme arginate. The heme oxygenase activity was increased 3.7 fold following heme arginate injection.
Kluczek & Kluczek (1985) 2wk to 3-months old calves were raised in stables with elevated air concentrations of carbon dioxide, ammonia, and H_2S (2-3 fold above control).	Blood serum concentrations of iron and transferrin saturation with iron were correlated negatively with the concentrations of carbon dioxide, ammonia and especially H_2S.
CIIT (1983 a,b,c) Mice and rats were exposed to 10, 30, 80 ppm H_2S for 6 hr/day, 5 days/wk for 90 days.	No effects on the hematopoietic system were observed.
Savolainen and Tenhunen (1982) Male rats given water containing sodium sulfite (Na2SO3) over ten weeks, for a total ingested dose of 5.6, 15.5, 28.9, and 41.0 mmol/kg for 1, 3, 7, and 10 weeks respectively.	At ten weeks, compared to controls, reduced cerebral RNA, reduced glutathione levels, and at seven and ten weeks, reduced brain and liver heme synthase levels were found.

Cited in US EPA (1981) Monkeys were exposed to 20 ppm H_2S for 90 days.	Changes in many blood parameters were observed.
Gavrilova (1980) Rats and cats were exposed to natural gas with varying H_2S concentrations (3-10 mg/m3).	Histochemical and immunological changes in the mesenteric lymph nodes were reported especially at the high concentrations. The number of secondary follicles was increased, macrophages were activated, and the number of pyroninophilic cells was increased. With increasing H_2S concentration, increased permeability of the vessels of microcirculation bed and sinus was seen.
Stolpe et al. (1976) Rats were exposed to 10 ppm H_2S for 41 days.	No difference in blood cells compared to controls was found.
Elebekova et al. (1976) Rats were exposed to 7 ppm H_2S daily (except Sundays) for 6 hr/day for 4 months.	Increased hemoglobin and leukocytes, and decreased erythrocytes were reported. Also, increased eosinophils and neutrophils, slight reticulocytosis, and decreased monocytes were found.
Malkov et al. (1976) 3-month old chicks were given H_2S of mineral origin (<0.2 mg/L) and iron at 5 - 7 mg/L in their drinking water from an artesian well.	Little effect was observed on hematological, bacteriological, histological and biological functions.
Nordstrom (1975) Calves were exposed to 20 ppm H_2S plus background ammonia (15 ppm) from the manure pack for 7 days continuously.	Sulfhemoglobin was not detected. Leukocytosis was observed in two calves. Other hematological abnormalities were not observed.
Barilyak and Vasil'eva (1974b) Rats were exposed to small concentrations of H_2S (specifics not provided)	In bone marrow cells, the number of aneuploid cells increased and structural chromosomal aberrations were found.

Misiakiewicz et al. (1972) Male wistar rats were exposed to a) 0.03 or b) 0.3 ppm CS_2 alone, c) a mixture of CS_2 (0.03 ppm) and H_2S (0.07 ppm), or d) a mixture of CS_2 (0.3 ppm) and H_2S (0.7 ppm) continuously (except for feeding) for 160 days in an inhalation chamber.	Urinary coproporphyrin concentrations in groups b), c) and d) increased 14, 57 and 100% from their initial levels, however the levels did not change in group a).
Solov'eva (1970) Rats were exposed daily by inhalation to 10 mg/m3 (7 ppm) H_2S for 6 hrs during 6 months.	Disturbances in carbohydrate metabolism of neutrophils, decreased phagocytic activity and levels of thrombocytes were observed.
Melnichenko (1968) Rats were exposed to 50 ppm H_2S and 300 ppm carbon monoxide for 4 hr/day for 6 months.	The number of erythrocytes increased 117% however no change in the hemoglobin content was found.
Bengtsson et al. (1965); Sallvik (1974)	H_2S has been suggested to be a possible cause for disorders of blood coagulation.
Sandage (1961) Rhesus monkeys were exposed to 20 ppm H_2S for 90 days in an inhalation chamber. No information on the number of hours per day or number of days per week was available.	Statistically significant changes in blood parameters at 90 days exposure over pre-exposure levels were found. Erythrocytes and amylase were increased, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) was decreased.
Sandage (1961) Mice were continuously exposed to 20 ppm H_2S for 90 days in an exposure chamber. Mice, monkeys and rats were also exposed to a mixture of indole (10 ppm), skatole (3 ppm), H_2S (20 ppm), methyl mercaptan (50 ppm).	Compared to baseline levels, increased leukocytes, hematocrit, hemoglobin, erythrocyte mean cell volume (MCV), erythrocyte mean cell hemoglobin, and a decreased number of erythrocytes were found in mice. Compared to controls at 90 days, increased leukocytes, reticulocytes, hematocrit, hemoglobin, MCV, mean cell hemoglobin were found. Exposure to the mixture produced significant sulfhemoglobin formation in all animals with mice most affected; significant lung and liver pathology and weight loss in exposed mice but not in exposed rats and monkeys and decreased stress endurance in rats.

Zburzhinskii (1961)	The erythrocytosis caused by H_2S in cats, rabbits, and mice was traced to reflexes from the carotid body and affected the spleen. Intravenous Na_2S caused a similar contraction of the spleen and a similar change in erythrocyte count.
Wakatsuki (1959) Rabbits were exposed continuously to 100 ppm H_2S, 300 ppm CS_2 or a combination of the two gases 30 minutes per day for 4 months. Clinical observations made during the study and continuing for 4 months post-exposure include general conditions, body weight, peripheral blood picture, serum calcium, blood specific gravity, total serum protein and serum protein fraction.	Rabbits exposed only to H_2S exhibited slight changes in oligochromemia, reticulocytosis, leukopenia, decreased pseudoacidophilic cells, relative lymphocytosis, and an increase in toxic granules, which recovered completely within 4 months. There was a decline in the specific gravity and albumin/globulin ratio of the blood. Rabbits exposed to a combination of the two gases exhibited more pronounced effects, and complete recovery was not observed.
Wakatsuki & Higashikawa (1959) Rabbits were exposed continuously to a) 100 ppm H_2S, b) 300 ppm CS_2 or c) a combination of the two gases 30 minutes per day for 4 months.	No significant differences in bone marrow activity were observed between the controls and groups a) or b). For the combined exposure, group c), there was a marked increase in bone-marrow cells. Severe hyperemia was seen in the spleens of the mixed gas group but no substantial changes were seen in the other two groups.
Ljunggren and Norberg (1943).	Hemorrhage in the lung alveoli was reported in rats exposed for 30-minutes to 1500 ppm methyl mercaptan.
Weedon et al. (1940) [cited in Toxcon, 1987] Mice were exposed to 63 ppm H_2S for 16 hours in a continuous flow chamber.	The mice were found to have, among other findings, massive lung hemorrhages, and distended stomachs with few moderate-sized hemorrhages.
Weise (1933) [cited in AB Health, 1983] 2 rabbits were exposed to 10 - 30 ppm H_2S in an inhalation chamber for 20 days. (No info given on hr/day or day/wk)	No effects on leukocyte or erythrocyte counts, or hemoglobin levels were found.

Table A11 Hematological System Effects: *In vitro* Studies

Reference / Study Design	Key Findings
Valentine et al. (1987) Inhibition of 22 erythrocyte enzymes was assessed in a system comprised of persulfides generated non-enzymatically from cystine in the presence of pyridoxal phosphate and mercaptopyruvate.	Of the 22 enzymes activities measured, thirteen were inhibited (hexokinase, 87-97%; phosphofructokinase 76 - 90%; aldolase <15 - 47%; glyderaldehyde-3-phosphate dehydrogenase, <15 - 31%; phosphoglycerate kinase, 57 - 67%; enolase, <15 - 28%; pyruvate kinase 79 - 92%; 6-phosphogluconate dehydrogenase, 42 - 70%; nucleoside phosphyrylase, 28 - 34%; adenylate kinase 64 - 93%; glutamine-oxaloacetic transaminase 31%; ribose-phosphate pyrophosphokinase 49%; AMP deaminase 65%). 9 were minimally or not at all affected (glucose phosphate isomerase, monophosphoglyceromutase, lactate dehydrogenase, glucose-6 phosphate dehydrogenase, glutathione reductase, acetylcholinesterase, adenosine deaminase and malic dehydrogenase). Partial to complete reversal of inhibition was observed in a subset.
Khan et al. (1987) Bovine red blood cells were exposed to various concentrations of sulfide, sulfite, and sulfate in vitro to determine their effects on various enzymes.	SOD activity was 78.7% of the control following exposure to 1.25 mM sodium sulfide. As the concentrations of sodium sulfide increased, enzyme activities decreased. The IC50 was approximately 10 mM for sodium sulfide, and just under 20 mM for sodium sulfite.
Tenhunen et al. (1983) Inhibition of haem synthesis enzymes by various sulphur species was studied in vitro.	Throughout the concentration ranges of 0.1 - 10 mmol/L, sulfide anion only inhibited haem synthase (Ki 3.4 mmol/L) while sulfite inhibited D-ALA-S (85% inhibition at 10 mmol/L) and thiosulfate inhibited haem synthase (Ki 27 mmol/L) and D-ALA-S (43% inhibition at 10 mmol/L). The in vitro inhibition is considerably higher than the exposures that the workers experienced.
Oivin (1943b)	Detoxification of H_2S in the organism is fundamentally oxidative binding into a pharmacologically inactive compound. This is a catalytic process involving heavy metals, particularly iron. Only protracted and massive doses of H_2S are able to reduce the activity of the system, which catalytically binds it in the blood.

Table A12 Hepatic System Effects

Reference / Study Design	Key Findings
Hayden and Roth (1988) Time-mated Sprague-Dawley rats were exposed to 105 mg/m3 H_2S for 7 hrs from day 7 of gestation until and including day 20 of gestation. The dams were sacrificed on day 21 of gestation for biochemical analysis of liver, brain and serum.	Liver and brain levels of DNA, protein, cholesterol, liver p450 and serum alkaline phosphatase were not significantly altered by H_2S exposure. However, cytochrome oxidase activity was significantly depressed by 10% in whole brain homogenates but was unaffected in hepatic tissue. Total alkaline phosphatase activity was suppressed by 36% in brain and 34% in liver when assayed in the absence of added magnesium.
CIIT (1983 a,b,c) Mice and rats were exposed to 10, 30, 80 ppm H_2S for 6 hr/day, 5 days/wk for 90 days.	No histopathological treatment related effects were found in the livers.
Sava and Giraldi (1981); [cited in Beauchamp et al., 1984] Guinea pigs were injected intravenously with 80 µg/kg (30ppm) sodium sulphide. Isolated segments of guinea pig gall bladder was also exposed to Na2S solutions ranging from 0.032 - 320 µg/mL.	Increased bile flow was reported in injected animals, which was blocked by papverine but not affected by atropine. Isolated gall bladder contracted upon exposure to sodium sulfide solutions.
Tansy et al. (1981) Rats were exposed to methyl mercaptan at 2, 17, and 57 ppm for 3 months.	Inference of liver damage is suggested by significant increases in total protein at all exposures, significant reduction in serum albumin, increased serum bilirubin and serum cholesterol in the 2 and 17 ppm groups.
Sava et al. (1980) [cited in Beauchamp et al., 1984] Sodium sulphide was given intravenously (40 µg/kg) to male Sprague-Dawley rats.	Significant reductions in bile flow were found and the antispasmodic, papaverine, blocked the effect whereas atropine failed to block the effect. This suggested that the response was not mediated by the parasympathetic system.
Beck et al. (1979), Poda (1966); [cited in NRC] Literature review	A synergistic action of ethanol with H_2S has been shown with male rats pre-treated with ethanol (0.33 and 0.66/kg body weight) and then exposed to 800 ppm H_2S. Ethanol increased the hazard of high-level, short-term exposure to H_2S. Those who had consumed alcohol within 24 hours of exposure were overcome by unusually small concentrations of H_2S.
Burnett et al. (1977) 221 people were exposed to H_2S by inhalation.	Increases in unspecified liver enzymes were found in several cases, however no baseline values were obtained.

NIOSH (1977)	Examination of high sulfur petrol refining workers showed and increased incidence of gall bladder and bile duct diseases including gallstones; which were stronger in workers with more than 5 years of service
Elebekova et al. (1976) White rats were exposed to 3.5 and 6.7 ppm H_2S daily except Sunday, for 6 hr/day for 4 months.	Reduced levels of liver glycogen, lower blood sugar, increased blood lipids and lower liver lipids were found at both exposure concentrations.
Nordstrom (1975) Two calves were exposed to 20 ppm H_2S plus background ammonia (15 ppm) from the manure pack for 7 days continuously.	No significant changes were observed for blood ammonia, urea nitrogen, uric acid, bilirubin, glucose, phosphorus, calcium, total protein, albumin, cholesterol, glutamic oxaloacetic transaminase, lactic acid dehydrogenase, or alkaline phosphatase.
Curtis et al. (1975) Crossbred pigs were exposed to 8.5 ppm H_2S continuously for 17 days.	No gross histopathological lesions were found in the livers.
Misiakiewicz et al. (1972) Male wistar rats were exposed to a) 0.03 or b) 0.3 ppm CS_2 alone, c) a mixture of CS_2 (0.03 ppm) and H_2S (0.07 ppm), or d) a mixture of CS_2 (0.3 ppm) and H_2S (0.7 ppm) continuously (except for feeding) for 160 days in an inhalation chamber.	Serum aspartate aminotransferase activity rose markedly in groups b) c) and d). Aminotransferase activity in each group (a-d) was 14, 64, 98 and 118%, respectively, greater than in the controls.
Bulatova et al. (1968) [cited in AB Health, 1988) 2465 high-sulphur petroleum refinery workers in two cities exposed to H_2S at unspecified concentrations were compared with 601 machine tool and 706 railway workers.	The incidence of cholecystitis (gall bladder disease), cholangitis (bile duct disease), and cholelithiasis (gallstones) and the number of workdays lost was greater in the oil refinery workers. The morbidity of oil refinery workers with over 5 years service was greater than that of those with less time in service. Contrast cholecystography showed that 56 of 474 cases of biliary dyskinesia in the oil-refining workers were of a hypermotor type and 12 were of a hypertonic type.

Martinez et al. (1963) 15 severe acute cases of H_2S poisoning were described. The first phase consisted of collapse or shock with hemoconcentration. The urine became acidic in all cases. Hematuria sometimes occurred. The second phase was more prolonged (7 - 10 days). The hemoconcentration diminished to more normal values, although leukocytosis persisted with neutrophilia in the more severe cases.	Values of serum glutamic-pyruvic transaminase (GPT) were initially normal but rose to moderately high levels after 5 days, and then gradually declined. Values for glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase were normal at the beginning of the second phase but rose to rather high levels after 3 days, followed by rapid decrease to normal after 6-12 days. Total blood lipids appeared unchanged. However, alpha-lipoproteins increased and beta-lipoproteins decreased, with maximal changes at 3-5 days. Values became normal about 10 days after the exposure to H_2S. No changes were observed in other blood protein fractions.
Sandage (1961) A 90-day continuous inhalation study of rats and mice exposed to 50 ppm methyl mercaptan.	Some hepatic effects (specifics not available) were observed in mice.
Falludi et al. (1955)	Rhodanese was purified 200-fold from beef liver. H_2S reduced the disulfide linkage of the enzyme.
Uchida et al. (1954)	The oxidase enzyme, isolated from rabbit liver, which oxidized p-hydroxyphenylpyruvate, was inhibited by H_2S.
Sheves (1949) The effects of pH, H_2S, and heavy metal salts upon liver proteinase and peptidase were studied.	H_2S depressed proteolysis, inhibited peptidases and did not affect activity of rat and rabbit livers, but in the presence of heavy metal salts, may cause considerable enzymic activity. The authors conclude that there is a proteinase in liver whose action is inhibited by copper, activated by H_2S and hence a papain-type enzyme.
Oivin & Oivin (1943) The effect of H_2S on tissue respiration was studied by Warburg's method.	Difficulties in the analysis were absorption of H_2S by the alkali in the side-arm vessel, rapid fall in the concentration in the fluid under investigation owing to oxidation and significant dissociation at pH 7.6 in Ringers solution. H_2S in concentration of 0.08 to 0.2 mM caused a small (17%) decrease in the respiration of liver slices. In lower concentrations, no certain or only a slight effect occurs from 0.2 mM to 0.8 uM.
Weedon et al. (1940) Mice were exposed to 63 ppm H_2S for 16 hrs.	Livers were pale and enlarged.

Table A13 Immune System Effects

Reference / Study Design	Key Findings
Claesson et al. (1989) Peripheral blood neutrophils were exposed to various concentrations of H_2S. Myeloperoxidase activity, phagocytosis and respiratory burst functions were evaluated.	A 1-hr exposure to 1 mM sulfide did not decrease their myeloperoxidase activity or their capacity to initiate a respiratory burst. Phagocytosis and bacterial cell killing in the presence of 1 mM sulfide was decreased only a minor amount. Myeloperoxidase was almost completely inhibited by 1 uM sulfide.
Setko et al. (1989) Effects of a single and chronic inhalation exposure (I month) to a complex mixture of H_2S (3 - 100 mg/m3; 2 - 71 ppm) containing natural gas (including hydrocarbon, mercaptan, and sulphur dioxide) on immunity in rats was studied.	A decline in humoral indicators of non-specific body resistance was found. Dose-dependent decreases in lysozyme, complement activity and bactericidal activity was found. Dose-dependent increases in beta-lysine concentrations in the serum were also found. Lysozyme concentrations were decreased also in the liver, kidney, lung and spleen.
Kluczek & Kluczek (1985) 2 wk to 3-months old calves were raised in stables with elevated air concentrations of carbon dioxide, ammonia, and H_2S (2-3 fold above control).	The resistance to respiratory and gastrointestinal infections of the calves was below those of control calves.
White (1982) Mercaptoethanol (30 - 300 mM) was employed under a variety of conditions (ph 7.5 - 8.8; 25 - 37C) to achieve reduction to intermediate levels.	Stepwise reduction of lysozyme concomitantly produces stepwise losses of enzymatic activity, until no activity for the fully reduced form could be demonstrated.
Rogers and Ferin (1981) After exposure of rats to 45 ppm H_2S for 2, 4 or 6 hours in a nose-only chamber, rats were anesthetized and challenged for 30-min. with a *S. epidermidis* -containing aerosol. Rats were sacrificed immediately following exposure, 3- and 6-hrs after challenge. Lung tissues were removed, homogenized, plated and the bacteria were grown on a selective media and counted.	Compared to controls, exposed rats had greater numbers of recoverable bacteria for all post-exposure durations. There was a clear dose-response relationship with the 4-hr H_2S exposure giving a 6.7- fold increase and the 6-hr exposure a 52-fold increase. The findings were attributed to an impaired antibacterial system of H_2S -exposed alveolar macrophages.

Gavrilova (1980) Rats and cats were exposed to natural gas with varying H_2S concentrations (3- 10 mg/m3).	Histochemical and immunological changes in the mesenteric lymph nodes were reported especially at the high concentrations. The number of secondary follicles was increased, macrophages were activated, and the number of pyroninophilic cells was increased. With increasing H_2S concentration, increased permeability of the vessels of microcirculation bed and sinus was seen.
Robinson (1979, 1980, 1982; cited in Toxcon, 1987) The effect of H_2S on rabbit alveolar macrophages cultured on gas permeable membranes was studied.	Phagocytic activity was reduced 96% after exposure to 60 ppm H_2S for 24 hours. Non-specific degenerative changes were also seen. Cell cultures exposed to 50 ppm H_2S were tested for phagocytic ability and viability after 24 hours recovery (Robinson, 1979). Relative to controls, phagocytic ability was 88% and viability was 88 - 94%. Exposure to 8 hours of 53.9 ppm H_2S had no effect on phagocytic ability whereas intermittent exposures produced a definite decrease.
Solov'eva (1970) Rats were exposed daily by inhalation to 2 - 10 mg/m3 (1.4 - 7 ppm) H_2S for 6 hrs during 6 months.	Disturbances in carbohydrate metabolism of neutrophils decreased phagocytic activity and levels of thrombocytes were observed following the 7 ppm exposure. No alterations in functional properties were observed at 1.4 ppm.
Fridyland (1959)	H_2S lowered the immunobiological activity of rabbits, pigeons, man and rodents against disease such as typhoid fever and anthrax.
Ludany et al. (1958)	In an *in vitro* system of rat leukocytes and *Staphylococcus aureus*, the addition of dilute solutions of H_2S decreased phagocytosis.
Babskii & Frolova (1946)	Guinea pigs fully sensitized to horse serum suffered fatal anaphylactic shock on intracardiac injection of the antigen. Administration of H_2S or Na_2S just before the antigen did not alter this outcome. The sensitized animals could withstand about twice the dose of sulfide (0.15 mg), which was effective in the controls, but sulfide clearly does not produce desensitization.

Table A14 Dermal Effects: Human and Animal Studies

Reference / Study Design	Key Findings
Beruashvili (1980) Guinea pigs were given daily baths in thermal water containing 10 - 12 mg H_2S /L (duration and amount not given).	Skin irritation was reported.
Benini and Colamussi (1969) [cited in Toxcon, 1987] Effects of 5 - 15 years exposures to 5 - 10 ppm H_2S (and SO_2) among 30 gasoline desulphurization plant workers are described.	Dermal symptoms suggestive of an allergic-type response are reported. Nine workers showed dermal effects, 4 with papulopruritis and erythrematosis-wheal-like lesions and 1 presented a syndrome of cutanea inveterata, pannicolopatia of the Besneir-Boek-Schaumann type, and a miliary sarcoidosis of the lung. Two of the 5 were sensitive to solution of H_2S in water as a patch test. Respiratory, gastrointestinal and ocular symptoms were also increased in exposed workers compared to controls.
Oivin (1943)	The skin of man and normal animals is permeable to undissociated H_2S and practically impermeable to sulfide ions. During electrophoresis with maximal current there is no resulting penetration of sulfide ion into the organism. For penetration by ions, damage of the horny layers of the skin is required.
Aves (1929) H_2S poisoning associated with oil exploration in Texas is reviewed.	The gas has a noticeable effect on minor skin wounds. At low concentrations, H_2S produces skin abrasions and lacerations, which fail to heal unless a gas tight dressing is applied.
Schutze (1927) Healthy full-grown cats were placed in a specially designed gas chamber in such a way that pure air was breathed, but the skin of the rump and extremities was exposed to the gas under investigation.	When the arms were exposed for 60 min to 100% H_2S, the skin was darkened, there were itching spots, red blotches and after several hours, erythema developed.

Table A15 Periodontal Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Tonzetich & Johnson (1986) Suspensions of rat-tail tendon collagen were reacted with 35 H_2S for 4 days at 4C and fractions were analyzed by gas chromatography.	All of the H_2S was absorbed - 47% was found in the supernatant, 53% associated with the cross-linked product and 2.7% associated with neutral salt-soluble protein product.
Johnson & Tonzetich (1985) Type I acid soluble collagen was exposed to air mixed with 10 nmol of ^{35}S-labelled 99.82% dimethyl disulfide/0.18% CH_3SH or with a similar mixture containing H_2S instead of CH_3SH.	All of the CH_3SH and H_2S were absorbed from the headspace within 48 hrs of incubation.
Ng and Tonzetich (1984) Porcine sublingual mucosa was placed in a permeability chamber apparatus and various H_2S or CH_3SH solutions were applied.	Permeability of porcine non-keratin zed sublingual mucosa is increased in a dose-dependent manner by up to 75 - 150% after 180 minutes after H_2S was applied at concentrations of 1.5 - 150 ng/ml. Methanethiol was similarly effective.
Bernardini et al. (1980) Medical investigation of 87 methane desulphurization workers was undertaken, with an emphasis on chronic intoxication by H_2S.	Significant prevalence of erosive lesions of dental crowns was found.
Blanchette and Cooper (1976) Concentrations of H_2S, CH_3SH were measured in the breath of 10 subjects over 6 - 10 days.	The H_2S concentration range was 65 - 698 ppb, and 10 - 188 ppb for CH_3SH. Previous work suggested that concentrations above 118 ppb H_2S and 25 ppb CH_3SH became objectionable.

Table A16 Effects on Gastrointestinal Mucosa

Reference / Study Design	Key Findings
Hsu (1980)	Carboxypeptidase A is inhibited by cysteine and sulfides.
Bernardini et al. (1980) Medical investigation of 87 methane desulphurization workers was undertaken, with an emphasis on chronic intoxication by H_2S.	Significant prevalence of gastrointestinal complaints was found.

Table A17 Ophthalmic Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Luck and Kaye (1989) Six men preparing sheep intestine for sausage skin were referred to the hospital after acute onset of painful, red eyes while at work.	The only chemical used in the process was salt. The barrels in which the tissues were stored were undergoing anaerobic decomposition, visible by the foamy head. The gas was believed to be due to hydrogen sulphide based on blackened zinc acetate test strips and no alkaline gases by litmus test. Eye examination showed bilateral blepharospasm, photophobia, and lacrimation, intense conjunctival injection, and superficial punctate corneal erosions.
Alberta Environmental Center (1986) Rats were exposed to 40 ppm H_2S for 6-hours.	Rats were agitated, showed a moderate degree of hypoaesthesia (a reduced perception of touch), panting and lacrimation for a period of 2 hours after.
Arnold et al. (1985) A retrospective study of workers who submitted WCB claims for H_2S poisoning.	18% had developed conjunctivitis, which persisted for several days in some cases.
Audeau et al. (1985) Description following acute H_2S exposure.	Stinging in the eyes was reported.
CIIT (1983 a,b,c) Mice and rats exposed for 90 days to 10, 30, and 80 ppm H_2S.	No effects were observed.
Haider et al. (1980) Guinea pigs exposed to 20 ppm H_2S for 1 hr/day for 20 days.	Eye irritation was observed.
Curtis et al. (1975) Crossbred pigs were exposed continuously to 8.5 ppm H_2S for 17 days.	No ocular lesions were found under microscopic examination of the eyes.
Nordstrom and McQuitty (1975) 36 calves were exposed to hydrogen sulphide (20 or 150 ppm) in enclosed chambers for 7 days of continuous exposure.	Photophobia and corneal opacities was observed at both levels as well as severe keratoconjunctivitis, clinical blindness, keratoconus (conical protrusion of the center of the cornea without inflammation), and incipient rupture of the eyeball at the higher exposure level. They concluded that a 7 - day exposure to 20 ppm resulted in corneal tissue damage, which could be considered permanent.
Hays (1972) Three lactating dairy cows were exposed to 20 ppm H_2S for a three wk period.	Slight lacrimation was observed.
Nessweetha (1969) Etiologic factors for "spinner's eye"' was studied in 6500 people were exposed to 15 -20 mg/m3 (11 - 14 ppm) H_2S for 4 - 7 hours	After exposure to ~10 ppm for 6 - 7 hours, eye irritation was reported. At exposures of ~14 ppm, symptoms developed after 4 - 5 hours. Night-shift workers had 41% incidence of conjunctivitis that the day-shift workers. Conjunctivitis was reported at H_2S concentrations below the allowable limit (15 mg/m3) when carbon disulfide was present.

Beasley (1963) A literature review of ocular effects of H_2S and case report of 5 men exposed to H_2S is given.	In the literature, lacrimation, photophobia, pain, chemosis, and eversion of the eyelids are reported following H_2S exposure, with recovery usually rapid within a few days. Rainbow vision is suggested as a warning of the onset of keratitis. Other symptoms reported include: smarting of eyes, sensation of grains of sands in the eyes, and haloes around artificial lights. Three of the 5 men developed delayed eye effects. Of the three men with detailed examination, 1 had orbital pain or ache, 2 had gritty sensation, all had hazy vision and rainbows in artificial light, and 1 had blepharospasm (involuntary contraction of eye muscles resulting in closure of both eyes).
Baikov (1963) 3 people (18 - 28 years) were exposed to 7-9 ppb H_2S for 5 minutes and repeated for 3 days.	Statistically significant increase in light sensitivity was reported following exposure to 9 ppb. No effect was seen at 7 ppb.
Duan (1959) 3 people were exposed to 7-9 ppb H_2S for 5 minutes.	Light sensitivity-related eye responses were increased.
Larsen (1944) [cited in Mehlman, 1994] A report of 50 workers building in a tunnel with H_2S (concentration varied with location; levels measured 29-132 ppm).	In 9 months, 163 instances of eye irritation were seen half an hour to several hours after workers descended into the tunnel; burning grainy sensation, and haloes were seen around lights. The symptoms were not pronounced in the tunnel, but increased when workers came out and included photophobia, lacrimation, broken blood vessels, and tiny blisters on the cornea, and occasionally a light cough irritation or the nose and pharynx, and nausea. The most exposed people developed conjunctivitis and blepharitis.
Legge (1934) 78 workers in the spinning department and acid cellars of an artificial silk factory in the Netherlands were surveyed in 1922.	H_2S concentrations were reported to vary between 20 and 35 mg/m3 (14 and 25 ppm). No information on exposure duration was available. Complaints of burning and smarting of the eyes was made by 25/78 workers. 32/78 workers complained of headache; > 25% experienced dizziness; 21/ 78 of loss of appetite; 20/ 78 of weight loss.
Aves (1929) H_2S poisoning associated with oil exploration in Texas is reviewed.	Irritation of the conjunctiva, lacrimation, and photophobia were seen.
Kranenburg and Kessener (1925) Eye injuries since 1921 caused by H_2S in beet sugar mills and rayon factories in the Netherlands were surveyed.	Eye damage was found in 95 cases where sewage polluted water had been used to wash beets. The symptoms included pain, photophobia, epiphora (increased tears), inability to open the eyes, and headaches. Levels of H_2S associated with conjunctivitis were 20 - 35 mg/m3 (14 - 25 ppm). Chronic exposure to similar levels also caused headache, dizziness, fatigue, nausea, poor appetite, and loss of weight.
(Ramazzini, 1713 [cited in Reiffenstein et al., 1992) Literature review.	Eye irritation was one of the first reported toxic effects of H_2S. The moist mucous surface of the eye is irritated by H_2S, which in severe cases, becomes ulcerated and scarred producing permanent visual impairment.

Table A18 Other Cellular Effects

Reference / Study Design	Key Findings
Ranus et al. (1985) Pigs were exposed to CO2, NH3 and H_2S at concentrations of >= 4005, >= 82.3 and >= 14.6 ppm respectively.	Compared to control pigs exposed to <= 3397, 82.3 and <= 7.7 ppm, CO2, NH3 and H_2S respectively, the concentrations of Ca, P, Mg, and Na in blood serum and erythrocytes and K in erythrocytes were below controls and K concentrations in blood serum were above controls
Sandmeyer (1981)	Excretion fluids (specifics not provided) containing erythrocytes and protein were also reported following acute exposures to methyl mercaptan
Bergstermann & Lummer (1947)	H_2S does not inactivate succinic dehydrogenase of muscle homogenate in the complete absence of air. The oxidation products formed in the presence of air (polysulfides, colloidal S and others) do inactivate the enzyme. The activity is restored by addition of cysteine.

Table A19 Nervous System Effects: Human Studies

Reference / Study Design	Key Findings
Wasch et al. (1989) Case report of three patients exposed to H_2S in separate unrelated incidents. The patient that had not lost consciousness was a soil inspector at a geyser/geothermal energy plant and was heating two 4.5 kg soil samples in convection and microwave ovens inside a trailer. After 30 minutes of heating, he noticed a rotten egg smell. Burning irritation of his face, eyes, and nose, dizziness, shortness of breath and electrical type feelings radiating down both arms and hands were experienced.	One of the three patients had not lost consciousness. This patient had a slightly abnormal EEG due to bilateral low-amplitude theta slowing at the hospital. On follow-up 9 months later, this patient continued to have parathesias of both arms, visual blurring, diminished short-term memory, and mental slowing. EEG was mildly abnormal with mild suppression of the background rhythm in the left frontotemperoal region. Further follow-up revealed moderate to severe deficits in sustained attention, concentration and visual spatial memory, and severe depression and anxiousness. P-300 event-related potentials were delayed in all three patients. In all three cases, attention and concentration were most severely impaired with lesser degrees of impairment in cognitive, memory, psychomotor, and perceptual abilities. Persistent neurological symptoms and neuropsychological testing indicated that all three patients developed persistent cognitive impairment.
Gaitonde et al. (1987) Case report on a 20-month old child living beside a continuously burning flare from a colliery for about a year. Three months prior to the child's presentation, the emissions had been monitored for 4 months and the maximum recorded level in the family's housing unit was 0.6 ppm H_2S, although officials acknowledge that the levels may at times have been higher.	The previously normal child had developed abnormal eye movements, progressive involuntary movements of the whole body with frequent falls, and on admission to hospital had marked and unsteady or uncoordinated trunk movements, involuntary movement with fine, disorganized and random movement of the extremities that was repetitive, slow and writhing, abnormal muscle tone, and could not stand. A scan showed abnormalities in the basal ganglia and surrounding white matter. The infant's condition improved spontaneously shortly after hospital admission. Rare metabolic diseases including mitochondriopathies were ruled out. The authors suggested that the infant's long-term exposure to hydrogen sulphide produced a temporary mitochondriopathy secondary to exposure. Other flare gases were undoubtedly present which confounds the association.
Arnold et al. (1985) A review of the medical records of 250 workers who were 'knocked down'.	More than half the workers (52%) presented with unconsciousness as the first sign of exposure. At the accident site 13.6% reported disequilibrium (loss of dizziness of balance), 8.8% at the hospital and 1.6 at the physician's office. Other neurologically associated complaints such as headache (8.8% at accident site; 3.6% at physicians office; 18% at hospital) and nausea (11.2% at accident site; 2.4% at physicians office; 14.8 % at hospital) were commonly seen. Twenty-eight workers (8%) exhibited neuro-psychological effects such as agitated behavior and amnesia following exposure. Five workers (2%) suffered convulsive episodes at the site. Computerized Axial Tomography was done on 4 workers who had been rendered unconscious for varied periods of time. One scan showed an abnormality while no other significant abnormalities were noted on the other three scans.

Arnold et al. (1984) A review of the medical records of 250 workers who were 'knocked down'.	Cerebral congestion and edema was found in 5 of 7 fatal cases from H_2S exposure.
Kangas et al. (1984) 81 pulp and paper workers were studied. The gas concentrations varied from 0- 20 ppm for H_2S, 1- 15 ppm for methyl mercaptan and dimethyl sulfide, and up to 1.5 ppm dimethyl disulfide.	Compared to matched controls, workers exposed to greater concentrations of H_2S, methyl mercaptan and dimethylsulfide experienced more chronic or recurrent headaches, and a decreased ability to concentrate. Symptoms of restlessness, lack of vigor, and more sick days and days away from work were also found more often among the exposed but the differences were not statistically significant,
Moden et al. (1983); Landrigan and Miller, (1983)□In 1983, 949 cases of illness were recorded at hospitals during a 2-wk period that appeared to be triggered by H_2S odour escaping from a latrine in a schoolyard in West Bank, Israel.	H_2S was detected at the site of the first outbreak at concentrations of 40 ppb and in a follow-up site visit ranging from 16 - 50 ppb in a latrine located 10 m from the main schoolbuilding. Concentrations in a subjacent pit ranged from 200 - 350 ppb. Methane (150 ppm) and hydrocarbons (250 ppm) were also detected. The main complaints were headache, dizziness, abdominal pains and blurred vision and were more severe and longer lasting in a subset of schoolgirls. Thirteen percent of cases reported smelling an unusual odour before they became ill; 5 percent described it as rotten egg odour.
Ando (1982) The long-term clinical courses of two cases of H_2S poisoning are described.	The chief residual symptoms were headache, heaviness of the head, visual disorders, hypertension, insomnia, disturbances of memorization and thinking, and impairment of intelligence. Computerized tomography showed slight dilation of the ventricles and slight cortical atrophy. Cerebral angiography revealed remarkable distortion and coiling of the cerebral artery in one case.
Matsuo and Cummins (1979) Case report of a 45-year old worker exposed to H_2S under high pressure. The worker was immediately rendered unconscious, and after respiratory assistance was instituted, spontaneous respiration soon resumed.	The worker, however remained comatose, had increased tonus with extensor spasms and Babinski's signs (loss or diminished Achilles tendon reflex). CT scans revealed bilateral symmetrical lesions within the cerebral hemispheres. The man remained in a chronic vegetative state and died about 5 weeks after exposure. The authors argue that systemic hypotension rather than a specific type of hypoxia plays a crucial role in the pathogenesis of such focal brain lesions.
Hirasawa (1976) Balance was assessed using a multipoint XY tracker and pedoscope in an H_2S -exposed individual with eyes open and eyes closed. (Conditions of exposure were not given).	Compared to the pattern of a normal subject, the pattern for the H_2S -exposed individual showed a marked tendency toward increased sway in the anterior-posterior direction.
Schielerr (1973)	Increased incidence of mental depression, dizziness and blurred vision, nausea, loss of appetite were reported at H_2S concentration 0.08 - 0.1 ppm.

Krasovitskaya et al. (1970) The health of children who lived in a polluted area around an organic synthesis plant was evaluated. H_2S , CS_2, SO_2, SO_3, N oxides, phenol and dust were present in zones 500 - 10,000 m from the plant. The greatest contamination was with H_2S, N oxides and dust.	The maximum allowable concentrations were exceeded in 34% of the analyses. Children living in the polluted area showed a lag in physical development, an elevation in the percentage with anemia and chronic rhinitis, and more frequent disturbances in central nervous system functions.
Chertok (1968) Case reports of acute H_2S poisoning in 29 workers.	In cases of minor poisoning, headaches, vertigo, felling faint, difficulty breathing, nausea (sometimes vomiting) photophobia, ocular pain, a brief loss of consciousness and some convulsions as well as moderately expressed autonomic disturbances were observed. These patients soon felt better and returned to work in a few days. In moderate poisoning, all of the above-mentioned symptoms were more significant. Loss of consciousness lasted up to 30 minutes. Afterwards, patients complained of protracted headaches, weakness, apathy, and loss of appetite and pallor. The sclera were congested, the pulse had a tendency to bradycardia, with muted heart sounds and reduced blood pressure. In most, there is increased perspiration, with cold extremities, apathy, and drowsiness. There was slight leukocytosis. Severe states showed prolonged loss of consciousness (up to 2 days), respiratory system disorders, with subsequent retrograde amnesia, and impaired intelligence.
Poda (1966) The effects of 174 H_2S exposed heavy-water plant workers from two plants were described. The normal maximum working concentration limit voluntarily adopted at the plants was 10 ppm for an 8-hr day.	A survey of the signs and symptoms reported by 123 of the workers showed, in decreasing order of frequency: weakness, nausea, dizziness, headache, nervousness, burning or watery eyes, clinical shock, gastrointestinal upset, vomiting, elevated blood pressure, dyspnea, sweating, cyanosis, flushed face, abdominal cramps, flatulence, arm and leg pain, rigidity, irrational and combative behavior or returning to consciousness, twitching, and frothy sputum. The most significant sign was clinical shock. In at least 27 workers, symptoms, including nervousness, headache, nausea, insomnia, weakness, cough, eye irritation, throat irritation, and soreness of the neck and shoulders, persisted for more than 4 hours. Workers who had consumed alcohol in the 24 hours prior to exposure were affected at lower H_2S concentrations.
Indiana Air Pollution Control Board (1964) Literature review	Color perception threshold was affected by H_2S in the range of 3 ppb. Increased incidence of mental depression, dizziness and blurred vision associated with 0.12 mg/m3 (86 ppb) H_2S. Increase incidence of nausea, loss of sleep, shortness of breath, and headaches following chronic exposure at 0.45 mg/m3 (0.32 ppm) H_2S. Increased incidence of decreased corneal reflex (convergence and divergence) after chronic exposure at 1.0 - 10 mg/m3 (0.7 - 7 ppm).
Loginova (1957) [cited in Toxcon, 1987] Effects of chronic H_2S exposure (4 - 210 ppb) in a mixture with CS2 and SO2 are described.	Headaches, weakness, nausea, vision problems and higher general morbidity rates in those households with greater than 36 ppb H_2S.

Ahlborg (1951) Symptom prevalence of shale oil workers was compared to controls.	72% of 459 workers usually exposed to 20 ppm H_2S or more complained of one of more of the following symptoms: fatigue, somnolence, lack of initiative, decreased libido, loss of appetite, headache, irritability, poor memory, anxiety, dizziness, itching, eye irritation, respiratory tract irritation, gastrointestinal disorders, insomnia and backache. Acute higher exposures gave rise to more severe, often neurological problems. These included: signs of cerebral and extrapyrimidal damage, facial paralysis, prolonged reaction time, absent of abnormal reflexes at both cranial and spinal nerve levels, poor memory for recent events, depression, either timidity or fierceness, and with an epileptic-like seizure. Sequelae of acute H_2S exposure occurred even when the affected individual had not lost consciousness. In one person, gastritis persisted for 1 year after exposure at unspecified concentrations. Another worker developed problems maintaining equilibrium 6 months after acute exposure to H_2S at an unspecified concentration. The disturbance of equilibrium still persisted after 3 years.
Sarai (1936) The amounts of H_2S, SO_2 and CS_2 in the air of 2 factories were detected. Symptoms produced by the gases are described.	Eight patients with mental symptoms due to CS_2 mostly showed symptoms of dementia praecox and of manic-depressive insanity. Recovery followed 10 days to 1-2 months after withdrawal from the factory. One patient showed neurasthenic symptoms for months.
Legge (1934) 78 workers in the spinning department and acid cellars of an artificial silk factory in the Netherlands were surveyed in 1922. H_2S concentrations were reported to vary between 20 and 35 mg/m3 (14 and 25 ppm). No information on exposure duration was available.	Complaints of burning and smarting of the eyes was made by 25/78 workers. 32/78 workers complained of headache. > 25% experienced dizziness 21/ 78 of loss of appetite 20/ 78 of weight loss.
Aves (1929) H_2S poisoning associated with oil exploration in Texas is reviewed.	Chronic exposure presents only an occasional incidence of nervous depression akin to shell shock.

Table A20 Nervous System Effects: Animal Studies

Reference / Study Design	Key Findings
Hannah et al. (1989) Pregnant rats were exposed to 75 ppm hydrogen sulphide for 7 h/day from day 5 postcoitus until day 21 postnatal in a 90L capacity chamber. Cerebellum and cerebral cortex amino acid levels were measured at 7, 14, and 21days postnatal.	Taurine levels were significantly higher in both cerebellum and cerebral cortex at 7 and 14 days post-natal however declined to control levels by day 21. Cerebellum levels of aspartate, glutamate and GABA were significantly reduced below control levels by day 21. Glycine levels did not differ from controls throughout the study in the cerebellum and cerebral cortex. Most of the cerebral cortex amino acid levels were altered for at least one period. Aspartate was reduced 40% on day 7 but increased to 80% of control value by day 14 and day 21. Glutamate was reduced to 80% of control values on day 7 and gradually climbed to control levels by day 21. GABA levels declined from control levels on day 7 to about 70% of control values on day 14 and 21.
Kombian et al. (1989) Rats were injected intraperitoneally with 15 mg/kg NaHS. Using a push-pull perfusion technique, neurotransmitter levels were determined.	No changes in brainstem glutamate, aspartate, glycine, or gamma amino benzoic acid levels were found. However, a delayed decreased in the release of glutamine to 61% of control was observed. At 3 µg/mL NaHS, near physiological levels, there was a 62% decrease in the glycine level when compared to controls, which could lead to unopposed excitatory events and loss of respiratory drive.
Reiffenstein (1989) Intraperitoneal injections of 10 or 30 mg/kg NaHS (corresponding to the LD30 and LD99) were given to rats. To test the effects of H_2S on the rates of spontaneously firing neurons, an in vitro hippocampal slice model and in vivo iontophoresis of single hippocampal pyramidal cells was evaluated.	Within two minutes of injection, there were significant increases in brainstem neurotransmitters: aspartate, glutamate, taurine, gamma amino benzoic acid, and alanine. No changes were found in the cerebral cortex, striatum and hippocampus. At the low dose, aspartate and glycine levels were decreased in the cerebellum and glutamine was elevated in the brainstem. Only 5-hydroxytryptamine and dopamine levels were increased in the brainstem, whereas epinephrine and norepinephrine levels were increased in the hippocampus and striatum.
Warenycia et al. (1989a) Intraperitoneal administration of sodium hydrosulfide at doses corresponding to 0.66 and 2 X the LD50 (10 and 30 mg/kg) to rats.	Significant increases in regional catecholamine levels were present in the brain. No changes were detected in the catecholamine levels in the cortex, however, significant increases of dopamine (140% of control) were found in the cerebellum at the high dose level. Within the hippocampus, brainstem and striatum, dose-dependent increases were found for both noradrenalin and adrenalin, however dopamine was increased in a dose-dependent manner only in the brainstem. Inhibition of MAO ex vivo could be demonstrated at concentrations above 20 µM, with an IC50 of 39.1 µM. The authors suggest that the increased catecholamine levels may be due to MAO inhibition, DBH or phenylethanolamine-N-methyl transferase activation.

Hayden and Roth (1988) Time-mated Sprague-Dawley rats were exposed to 105 mg/m3 H_2S for 7 hrs from day 7 of gestation until and including day 20 of gestation. The dams were sacrificed on day 21 of gestation for biochemical analysis of liver, brain and serum.	Liver and brain levels of DNA, protein, cholesterol, liver p450 and serum alkaline phosphatase were not significantly altered by H_2S exposure. However, cytochrome oxidase activity was significantly depressed by 10% in whole brain homogenates but was unaffected in hepatic tissue. Total alkaline phosphatase activity was suppressed by 36% in brain and 34% in liver when assayed in the absence of added magnesium.
Reiffenstein (1988) Rats were injected with 30 mg/kg NaHS intraperitoneally.	Epinephrine and norepinephrine and dopamine were higher than controls in the brainstem.
Arthur D. Little, Inc. (1987) [cited in US EPA, 1993] Rats were administered by oral gavage solutions containing 1, 3.5, or 70 mg/kg/day H_2S, once daily, 7 days/wk for 89 days.	Clinical signs of restlessness (males) and salivation (females) were observed in the lowest exposure group and increased in a dose-related manner. Significant increases in mortality occurred in the high dose.
Alberta Environmental Center (1986) Rats were exposure to 40 ppm H_2S for 6 hours.	Rats were agitated, showed a moderate degree of hypoaesthesia (a reduced perception of touch), panting and lacrimation for a period of 2 hours after.
Haider and Hasan (1984) Guinea pigs were exposed alternately to 20 ppm H_2S and 10 ppm SO2 daily for 1 hour/day for 30 days in an exposure chamber. On day 30 of exposure, the cerebral hemisphere and cerebellum and brainstem was removed and analyzed for total lipids, cholesterol, phospholipids, free fatty acids, and lipid peroxidation.	Significant reductions of total lipids in cerebral cortex, basal ganglia, brainstem, and spinal cord were found, whereas the cerebellum had only a 3 % decrease. Phospholipids were increased in the cerebellum and brainstem. Cholesterol was significantly decreased in the cerebral cortex, basal ganglia, cerebellum, brainstem and spinal cord. Elevated levels of free fatty acids were found in cerebral cortex and basal ganglia and decreased levels found in cerebellum, brainstem and spinal cord. A large decrease in esterified fatty acids was observed in basal ganglia, cerebellum, and spinal cord. Lipid peroxidation and lipase activity were significantly increased in various regions of the brain and spinal cord. The lipid reductions were associated with an 18% increase in malonaldehyde in the central hemisphere, suggesting coincident lipid peroxidation.
Aitbaev et al. (1984) White rats were exposed to 0.89 - 9.94 mg SO2/ m3 (0.36 - 4.0 ppm) plus 0.83 - 10.09 mg H_2S /m3 (0.6 - 7.2 ppm) for 4 hr/day and 5 days/wk for 4 months by inhalation.	Concentration dependent inhibition of oxygen consumption and in the activities of cytochrome oxidase and succinate dehydrogenase in the lungs, cerebral cortex, heart, liver, kidneys and skeletal muscles was observed.
CIIT (1983) Rats were exposed to 10, 30, or 80 ppm H_2S 6hr/d, 5 d/wk for 90 days. Clinical hematologic, urinary, and serum chemistry parameters, and detailed necropsy and microscopic examinations were done. Also, detailed neurologic and ophthalmologic studies were conducted.	A depression in brain weight in rats exposed to 80 ppm versus that of controls was observed.

Dahme et al. (1983) [cited in US EPA, 1993] Eight cattle survived 18 hours to 10 days after H_2S exposure (concentration not given). The brains were examined histologically.	Spotty regions of neuronal necrosis with vascular proliferation and gliosis in the basal ganglia were found. Up to 60 hours after intoxication, bilaterally symmetrical lesions (characterized by eosinophilic neuronal necrosis, astrocytic edema and low-grade white matter edema) were seen in the dorsal neocortex and, to a lesser degree, in the hippocampus, the lateral geniculate nucleus, the caudate nucleus and the cerebellar Purkinje cell layer. After 10 days post-exposure, the lesions had progressed to laminar necrosis with resorption of necrotic tissue by macrophages. The lesions seen were similar to those seen in systemic hypoxia.
Savolainen and Tenhunen (1982) Male rats were given water containing sodium sulfite (0.9g/L) of 1 - 10 weeks. They ingested a total dose of 41 mmol sulfite per kilogram of body weight.	Brain glutathione was above the control at the first week and below the control at 10 weeks. Brain RNA content was below the control at 10 weeks and brain and liver heme synthase were below the control after 7 weeks.
Haider et al. (1980) Guinea pigs were exposed to 20 ppm H_2S for one hour per day for 11 days.	Reduced concentrations of cerebral hemisphere and brainstem total lipids (19- 34%) and phospholipids (11 -21%) were found. Increased lipid peroxidation (18%) was also observed in the cerebral hemisphere; an 18% increase in malonaldehyde in the central hemisphere, suggesting coincident lipid peroxidation.
Elovaara et al. (1978) Adult female mice were exposed to 100 ppm H_2S for 2 hours.	Compared to controls, decreased labeled leucine incorporation in cerebral protein and myelin 24 and 48 hours after exposure concomitant with a decrease lysosomal acid proteinase activity was observed. The incorporation increased to control levels 72 hours after exposure however the lysosomal enzyme activity remained below the control at the end of the follow-up period.
Higuchi (1977) [cited in US EPA, 1993] Rats were exposed to 100 - 500 ppm H_2S (duration not given)	At 200 ppm, there was an immediate inhibition of discriminated avoidance response; at 300 - 500 ppm, the Sidman-type avoidance response was also inhibited in a dose-dependent manner.
Nordstrum (1975) Calves were exposed to ammonia (13 ppm) continuously for 7 days and then to ammonia (16 ppm) and hydrogen sulphide (20 ppm) gases in combination for 7 days of continuous exposure.	The calves were distressed and depressed. The signs of distress were restlessness, pawing, head-shaking, tail-switching, blowing through the nose, licking of the nose, and slobbering with the tongue protruding. Signs of depression were heads lowered and eyes closed. Photophobia and refusal to open eyelids were observed at 20 ppm H_2S.
Hays (1972) Mice and cows were exposed to 20 ppm (duration not specified). Goats were exposed to 50 and 100 ppm.	At 24 hours after exposure, mice exhibited decreased performance as indicated by decreased feed and water intake and decreased rectal temperature; cows were not affected by a similar exposure. Exposed goats had decreased performance shown by feed and water consumption. Goats exposed to 50 and 100 ppm for 4 days were generally stressed as shown by increased plasma cortisol and fever.

Pashchenko (1969) The effects of acute, sub-acute and chronic exposures to H_2S and methyl mercaptan (CH_3SH) on ATPase in brain, skeletal muscles, kidney, lung, heart and spleen in mice were studied. Acute exposures were to 570 - 714 ppm H_2S and 2000 - 2500 ppm CH_3SH (duration not given); sub-acute exposures were carried out 3 hr/day for 3 weeks at 72 - 144 ppm H_2S and 500 - 1000 ppm CH_3SH; and chronic exposures were daily for 3 hrs during 3 months at 29 – 43 ppm H_2S and 200 -300 ppm CH_3SH.	Acute H_2S poisoning decreased ATPase activity 26% in the lungs, and subacute H_2S poisoning decreased brain ATPase 14%. Acute CH_3SH exposure significantly decreased activity in the brain, lungs, and spleen and increased activities were found in the brain and spleen after chronic CH_3SH poisoning.
Kaminski & Mikolajczyk (1967) Rabbits were exposed to 71 ppm (0.1 mg/L) H_2S for 30 minutes daily by inhalation for 7, 10, and 14 days.	Histochemical examination of the cytoplasm of the motor cells of the anterior horns of the spinal cord showed enhanced activities of acid phosphatase, alkaline phosphatase, and DNAse II. DNAse II activity decreased slightly after exposures longer than 7 days.
Kamnev & Lyutikova (1966) Mice were exposed to a mixture of hydrocarbons from petroleum cracking and H_2S at the maximum permissible concentrations (10 ppm) for 6 hrs/day for 10 - 125 days. The brains were examined histologically.	There was hyperemia of the brain, migration of leukocytes from the vessels with infiltration around the vessels, deformation of the nerve cells of the cortex, appearance of shadow cells, especially in the nuclei of the brainstem and the thalamic-hypothalamic area, changes in the glial elements, the presence of cells of the microglia in unusual places, the appearance of dendritic cells, the formation of microglial nodes, and intensified activation of the oligoglia.
Lund and Wieland (1966) [cited in Mehlman, 1994; and in ACGIH Documentation for H_2S] Monkeys were exposed to 500 ppm H_2S for 22 to 35 minutes.	Each of the monkeys lost consciousness in about 15 minutes. Examination of the brains showed damage in the basal ganglia, and increase in neuroglia, and a decrease in purkinjie cells in the cerebellum.
Filippova (1963) The toxicity of cracking gas with and without H_2S, or without saturated hydrocarbons was studied.	Mean swimming time for animals exposed to H_2S was 71.2 minutes, for H_2S plus butylene, 25.7 minutes, for whole cracking gas, 31.5 min. The authors conclude that a higher toxicity exists from a mixture of hydrocarbons with H_2S than from any components of the mixture and that in production facilities where H_2S is mixed with lower hydrocarbons, a maximum permissible concentration of 3 mg/m3 is recommended.
Fyn-Zhuy (1959) Male rats were exposed to 0.2 or 10mg/m3 H_2S 12 hrs/day for 3 months.	Animals had lower chronaxy of flexors at both exposures. The inhalation of H_2S in both concentrations for 3 months caused changes in the functional states of the cerebral cortex. The 10mg/m3 concentration caused changes in dendrite morphology (prickled, thickened and swollen), and dendrite spikes in the brain cortex to disappear.
Stern & Lokchina (1927)	Poisoning of rabbits by CO, H_2S or HCN causes a lowering of resistance of the hemato-encephalic barrier toward colloids such as trypan blue and Congo red, without altering its resistance against crystalloids such as iodides and ferrocyanides.

Table A21 *In vitro* **Neurochemical Effects**

Reference / Study Design	Key Findings
Perez-Clausell and Danscher (1986) Sodium sulfide was injected intracerebrally in Wistar rats to allow zinc sulfide accumulations to form in vivo. The fate of the zinc sulfide was followed using different survival times.	After 30 minutes, zinc was found in boutons located in synaptic vesicles, later in vesicles and in the synaptic clefts. After long survival times (48 hours), zinc was located solely in the extracellular space. After injections of sodium sulfide into the hippocampal region, 'locomotor depression' was repeatedly observed in the animals.
Dooley and Cote (1984); Messerschmidt (1998) Sablin and Ramsey (1998); Ramsey and Sablin (1999)	Sulfide irreversibly inactivates beef plasma amine oxidase, which is a member of the copper-containing amine oxidase family. The data strongly suggested that enzyme-bound copper is the site of action for inhibition by sulfide. The active site mechanism is not well understood, however recent work suggests that in addition to the active site flavin group, another redox-active group is present, likely a disulfide group.
Waller (1977)	Methanethiol inhibits mitochondrial respiration (50% inhibition at 72 nmol/mL), which was found to be partly reversible, apparently by reacting with cytochrome c oxidase.
Foster et al. (1974) and Quarforth et al. (1976)	Evidence was presented that methanethiol reversibly inhibits rat brain Na+, K+ -ATPase, and that the inhibition was concurrent with stimulation of the associated K+ -dependent phosphatase.
Kosmider (1966)	The activity of alkaline phosphatase of blood serum and brain tissue was significantly decreased in humans and rabbits after a 20-minute exposure to H_2S. $MgCl_2$ recovered the enzyme activity.

Table A22 Renal Effects of H_2S Exposure

Study Design / Reference	Key Findings
Audeau et al. (1985) Case report of 4 workers exposed to H_2S in pelt room.	For 2/4 workers, a trace of albumin was found initially, but later was normal. 1/4 workers had 0.3 g/l albumin and some granular casts in a mid-stream urine specimen.
CIIT (1983) Mice and rats exposed for 90 days to 10, 30, and 80 ppm H_2S.	No effects were seen.
Peters (1981) Case report of a worker exposed to H_2S (and likely SO_2) from a plugged drain trap cleaned with 90% sulphuric acid.	Urinalysis was normal except for proteinuria (1+) was found on admission to hospital.
Dreisback (1980) Reference text.	Proteinuria and hematuria may be a feature of hydrogen sulphide poisoning.☐
Burnett et al. (1977) 221 people were exposed to H_2S by inhalation.	Blood urea nitrogen concentration, recorded in 23 cases, ranged from 11 to 170 mg/dL with a mean of 24 mg/dL (Normal range 7 - 18 mg/dL). A trace of protein was noted in the urine of 12 patients and larger amounts in 20; at the time of discharge, protein was still detectable in the urine of 4 patients.
Aitbaev et al (1976) Rats exposed to 7 ppm H_2S for 6 hr/day, 5 d/wk for 120 days	No change in kidney succinate dehydrogenase activity was found.
Kosminder (1973); [cited in Beauchamp et al. (1984)] Rabbits were exposed to 72 ppm H_2S for 1 hr/day for 14 days.	In renal tissue, staining intensity for succinic dehydrogenase and alkaline phosphatase decreased and acid phosphatase increased. The staining intensity decreased for ATPase in the cortex and increased in the medulla.

Mueller (1964) Adult albino rats and growing guinea pigs were chronically poisoned with H_2S administered for 10 hrs to 3 days. The animals were then killed with a massive dose of H_2S. Tissues were fixed in 70% ethanol saturated with H_2S, embedded in paraffin and stained by the cadmium-silver, cobalt nitrate and osmium methods.	The animals treated with H_2S showed much clearer staining by the sulfide silver method. Silvered zones in the cytoplasm were strictly localized and corresponded to the golgi fields shown by classical staining methods. The authors proposed that sulfide was fixed in vivo by heavy metals normally serving as catalysts in enzyme reactions. In renal tubular epithelium dense apical granulation was observed, possibly corresponding to areas of specific secretory function.
Sandage (1961) Animals exposed continuously to 20 ppm H_2S for 90 days	Kidney pathology was observed in 6% of test animals.□
Wakatsuki & Higashikawa (1959) Rabbits were exposed continuously to a) 100 ppm H_2S, b) 300 ppm CS2 or c) a combination of the two gases 30 minutes per day for 4 months.	Very mild kidney changes were seen in groups a) and b). In addition to the changes seen in a) and b), group c) also showed degeneration of the epithelium of the uriniferous tubules and presence of calcium deposits.
Weedon et al. (1940) Mice exposed to 63 ppm H_2S for 16 hrs	'Pale kidneys' were observed.
Helfors (1938) Case report of a man inhaling H_2S.	The man became dizzy, suffered chest constriction, irritation of throat, diffuse bronchitis, diarrhea and vomiting. The next day there was sugar in the urine and blood sugar was 126 mg%. The man died soon of angina pectoris.

Table A23 Reproductive Effects: Animal and Human Studies

Reference / Study Design	Key Findings
Saillenfait et al. (1989) Pregnant Sprague-Dawley rats were exposed to 50, 100 or 150 ppm H_2S on gestation days 6 - 20.	Exposure at 50 ppm had no effect on body weight gain or absolute weight gain (minus the gravid uterine weight). H_2S also enhanced the fetal toxicity of CS_2.
Derzhko and Barilyak (1986) Rats were exposed to H_2S for 4 hours (concentration not specified) on day 10 - 12 of pregnancy. Before H_2S exposure, rats were given 20 mg phenobarbital/kg or (glutamate, zinc sulfate, copper sulphate, iron lactate and ascorbic acid) at 50, 1, 0.5, 10, and 50mg/kg respectively.	No effects were found on the rats' bone marrow chromosomes following H_2S exposure. The number of chromosomes damaged in embryos was increased approximately 5-fold. Pre-treatment with phenobarbital increased the chromosome damage in the bone marrow cells. Administration of the glutamate and other substances prevented the chromosomal damage by H_2S
Hemminki and Niemi (1982) Information on spontaneous abortions was collected from the hospital discharge register; information on women and their families living in an industrial community was obtained from the population and housing census. Air pollution data was collected from the Institute of Meteorological Sciences.	Women employed in rayon textile jobs (10.3%) and paper products jobs (16.7%) had an increased rate of spontaneous abortions, compared to 7.7% in the total sample. The wives of men employed in transport and communication (15.8 %), in viscose rayon textile jobs (14.9%), and in chemical process jobs (28.6%) also had an increased rate of spontaneous abortion compared to 8.6% in the total sample. More spontaneous abortions were noted in all socioeconomic classes in areas where the mean annual level of H_2S was less than (7.6%) or more than 4 μg/m3 (3 ppb) (9.3%), however the difference was not statistically significant. No relationships were found between miscarriage rates and SO_2 or CS_2 concentrations.
Andrew et al. (1980) (cited in IRIS database) Female Wistar rats were exposed to 220 ppm H_2S for 3 hr/day, 5 days/wk on gestation days 1 - 18, 7 - 11, or 12 - 16. Male Wistar rats were exposed to 220 ppm H_2S for 3 hr/day for 1 week and then mated over a 10 - week period.	No effect on maternal toxicity, fertility, prenatal mortality or litter weight was observed however an increase in minor skeletal anomalies was observed. In the offspring of the exposed male rats, no exposure-related effects on fertility, corpora lutea, implants, or resorptions were found. There was a high incidence of wavy ribs in the exposed group throughout gestation.
Beruashvili (1980) White rats ingested thermal water containing 10 - 12 mg H_2S /L (duration and amount not given).	Depression of sperm counts in males and embryotoxic reactions in pregnant females were reported.

Barilyak et al. (1975) The effects of inhalation of H_2S plus CS_2 on embryonic development of rats were studied. The combined concentration was 10 mg/m3 although the actual individual concentrations were not given.	Prolonged exposure led to abnormalities of the genitourinary and bone systems, disturbances of embryonal blood formation and the ossification process, and several changes of the liver and kidney parenchymal cells in rat embryos.
Glebova (1950) [cited in Toxcon, 1987] Effects of chronic H_2S exposure (20 - 40 ppb) in a mixture with CS_2 and SO_2 are described.	Babies were poorly developed, underweight, listless, anemic, dyspeptic and more susceptible to infectious disease.
Barilyak and Vasil'eva (1974a) Female rats were exposed to 7 ppm H_2S for 70 - 90 days and then for the first 20 days of their pregnancy.	Decreased mitotic activity of embryonic hepatocytes and epithelial cells of the renal cortex was observed.
Vasil'eva (1973) [cited in NRC, 1981]	Continuous long-term occupational H_2S exposure may have had some adverse effect on the menstrual and reproductive functions of female employees.
Wakatsuki & Higashikawa (1959) Rabbits were exposed continuously to a) 100 ppm H_2S, b) 300 ppm CS_2 or c) a combination of the two gases 30 minutes per day for 4 months.	There were no significant testicular changes in the CS_2 group; diminished spermatogenic capability from seminiferous tubular atrophy was seen in the H_2S group. Spermatogenesis ceased entirely in the rabbits exposed to the gas mixture.
Hueper (1942)	A review of the effects of H_2S and other substances on male gonads is given.
Hofmann (1928)	Data obtained from studies of the effects of manure gas on farm livestock indicate that continuous long-term exposure to 1 ppm may cause decreased fertility and an increased incidence of abortion.

Table A24 Respiratory System Effects: Human Studies

Reference / Study Design	Key Findings
Dales et al. (1989) A cross- sectional study of residents living near a natural gas refinery compared various health outcomes with that of an unexposed control group.	In children, significantly increased odds ratios for persistent cough 1.31 [95%CI 1.14 -1.5]; for persistent phlegm 1.31 [95%CI 1.13 - 1.52]; and for wheeze, 1.18 [95%CI 1.03 - 1.35] was found. Also for both children and adults, an association between exposure and reduced pulmonary function was found.
Spitzer et al., (1989) A cross- sectional study of residents living near a natural gas refinery compared various health outcomes with that of an unexposed control group.	Compared to reference groups, an increased proportion of people living near a natural gas refinery were found to hay fever or allergic rhinitis, nasal symptoms and shortness of breath reported, and worry about general health.
Shusterman et al. (1989) The sulphur gases were below the detection limit of sensitive air monitoring equipment (0.1 ppb for H_2S; 0.02 ppb for mercaptans).	Increased frequency of headaches, upper respiratory tract irritation, and nausea among community members downwind of a hazardous waste site following the release of sulphur gases from settling ponds.
AB Health (1988) Literature Review	At concentrations of 50 ppm, irritation effects such as inflammation, dryness of the nose, throat, larynx and bronchi producing symptoms of hoarse throat, nasal secretions, cough, and shortness of breath, occur in humans within 30 - 60 minutes.
Nethercott and Holness (1988) Lung function was assessed in fifty sewage workers using a heat treatment sewage sludge process.	Workers reported 'influenza-like' symptoms, cough, sputum production, wheeze, sore throat and skin complaints. These workers tended to have reduced lung function, particularly in the area of the plant where the dried sludge was incinerated.
Deng and Chang (1987) Report of 6 cases of maintenance personnel cleaning hot-spring reservoirs overcome by H_2S.	Acute respiratory distress syndrome was found in 2 of the 6 cases, one of who was alert initially and could describe the whole incident, did not lose consciousness. However, he developed refractory respiratory failure and died 12 hours later.
Arnold et al. (1985) A review of the medical records of 250 workers who were 'knocked down'.	Increased respiratory symptoms are consistently reported following both high and low level H_2S exposure. Shortness of breath accounted for 23% of symptom complaints. Worsening of asthmatic symptoms, sore throat, cough, and chest pain were associated with relatively high hydrogen sulphide exposure. In 9 workers given pulmonary function tests, three showed an obstructive pattern.
Bhambhani and Singh (1985) Young male and female volunteers exposed to 5 ppm H_2S for 30 minutes	Coughing and burning throats were reported in some.
Arnold et al. (1984) A review of the medical records of 250 workers who were 'knocked down'.	Pulmonary congestion and edema was found in 6 of 7 fatal cases from H_2S exposure.

Dales et al. (1984) Cross-sectional study in the vicinity of natural gas refineries.	Children reported significantly more persistent cough, phlegm, and wheeze than individuals who lived in the control area.
Donham et al. (1982) Case reports of 6 workers exposed to emissions from liquid manure storage facilities.	Extensive pulmonary edema was found in 2/6 workers. One of the workers reported that he became 'unable to breathe' among other symptoms, was too ill to work for 3 days and for over two months he was 'short of breath and was just not feeling well.' Another reported that after noticing a 'peculiar odour', after a few seconds, had 'a very hard time getting his breath.'
Bernardini et al. (1980) Medical investigation of 87 methane desulphurization workers was undertaken, with an emphasis on chronic intoxication.	A high prevalence of chronic inflammation of upper airways was seen.
Biesold et al. (1977) An electron microscopic examination of lung tissue from a 7-year old boy that died after being exposed to H_2S from a latrine.	A severe alveolar and interstitial edema of the hemorrhagic type was found. Evidence of direct toxic effect on the endothelial and epithelial barrier of the alveoli was observed. Widespread damage to the squamous epithelium, partially denuding the basement membrane, and endothelial gaps were found often covered with microthrombi.
Holasova (1969) A comparison of the incidence of upper respiratory diseases was made between children living near an industrial plant and a control group living in a rural district.	The industrial plant was emitting 32 kg/hr H_2S and 55.5 kg/hr CS_2. The incidence of upper respiratory tract diseases was 10% higher near the plant as compared to the control group.
Katz (1962) In the geothermal area of New Zealand, natural steam for electrical power is obtained from deep wells.	The 500 - 1000 ppm H_2S in the wellhead product have caused no acute symptoms in a town near a 150-megawatt plant, although there were complaints of excessive fits of coughing, chest constriction and insomnia.
Frontczak (1961) Vital capacity was measured in 88 healthy individuals by means of a spirometer. Fifty healthy males were then exposed for 5 to 10 minutes in an atmosphere containing up to 20 mg/m3 H_2S.	Exposure to H_2S decreased lung capacity by an average of 274 cm3. No difference was observed in 16 % of the subjects and in 84%, vital capacity reductions varied from 100 to 500 cm3.

Table A25 Respiratory System Effects: Animal Studies

Reference / Study Design	Key Findings
Lopez, (1988b) Rats were exposed to 83, and 439 ppm H_2S for 4 hours and then examined.	A mild perivascular edema was found at 83 ppm. Many changes were observed following the 439 ppm exposure: severe but transient pulmonary edema and fibrocellular alveolitis in proximal alveoli, cytoplasmic blebs in the alveolar epithelium, increased numbers of mitotic figures in the bronchiolar epithelium, minor changes in the alveolar epithelium, and necrosis of the ciliated bronchiolar cells.
Hulbert et al. (Banff, 1988) Rats were exposed to H_2S (1 ppm 8 hr/day, 5day/wk for 5 wk) and then challenged with methacholine.	Evidence of increased bronchial reactivity or hypersensitivity in animals (2/10) with low level exposure; also found a reduced number of tracheal monocytes in rats exposed to 1 ppm H_2S (for the same duration) compared to controls and at 100 ppm, a severely altered ratio of ciliated to non-ciliated cells.
Lopez (1987, 1988b) Male rats exposed to 10, 200, or 400 ppm H_2S for 4 hours and examined at 1, 20, or 44 hours post-exposure	The nasal lavage fluid had increased cellularity at all exposure concentrations. At 1 hr post exposure, exfoliated epithelial cells were found and neutrophils were found at 20 hours post-exposure. Some histological changes were observed at 10 and 200 ppm.
Alberta Environmental Center (1986) 6-hour exposure to 40 ppm H_2S	Necrosis of the nasal epithelium was observed in these animals immediately following exposure. Eighteen hours following exposure, necrosis and neutrophils were observed in the nasal epithelium and 43 hrs after exposure, reparation of the nasal epithelium was underway. The most rostral and most caudal parts of the nasal cavity were considerably less affected than the intermediate parts. In the intermediate parts of the nasal cavity, the lateral aspects of the nasal turbinates had more severe necrosis compared to the medial aspects, especially the nasal septum epithelium. Mild pulmonary edema was observed in animals exposed to 6 hrs at 40 ppm H_2S immediately following exposure, but not at 18 or 42 hrs post-exposure.
CIIT (1983) Rats (F344) were exposed to 10, 30, or 80 ppm H_2S 6 hr/d, 5 d/wk for 90 days.	Clinically observed crustiness with ear tags, crusty nose, eye muzzle; lacrimation, and rales were reported.
CIIT (1983) Mice exposed to 80 ppm for 90 days at 6 hr/d, 5 d/wk	Inflammation of the nasal mucosa in the anterior segments of the nose was observed in 8/9 male and 7/9 female mice. The lesions were characterized as minimal to mild in severity affecting primarily the squamous portions of the nasal mucosa, but extending to areas covered by respiratory epithelium.
Curtis et al. (1975) Three crossbred pigs were continuously exposed to 0 or 8.5 ppm H_2S in an inhalation chamber for 17 days.	No significant changes in body weight gain and no histopathological changes in the respiratory tract (including the turbinates, trachea, and lungs) were observed.

Nordstrum (1975) 35 steer calves were exposed to 20 and 150 ppm continuously in enclosed chambers for one week.	Respiration was slightly increased in mice exposed to 20 ppm and reduced at 150 ppm.
Hays (1972) Goats were exposed to 100 ppm H_2S for 4 days.	A decrease in respiration rate was observed in the goats.
Misiakiewicz et al. (1972) Male wistar rats were exposed to a) 0.03 or b) 0.3 ppm CS2 alone, c) a mixture of CS2 (0.03 ppm) and H2S (0.07 ppm), or d) a mixture of CS2 (0.3 ppm) and H2S (0.7 ppm) continuously (except for feeding) for 160 days in an inhalation chamber.	Chronic inflammation of the lobular bronchi and bronchogenic inflammation of the lungs was present in groups b) and c). Chronic inflammation of the segmented bronchi was the predominant finding in group d).
Braginskaya et al. (1968); [cited in Gangolli, 1999) Wistar rats were exposed to 75 ppm H_2S for 20 - 60 min.	Slight congestion of the lungs was reported.
Sandage (1961) [cited in Toxcon, 1989] Rats exposed continuously to 20 ppm H_2S for 90 days.	Lung pathology observed in 33%of exposed animals, compared to 17% of controls; lung abscesses, in mice that died during the test period, and bronchopneumonia were also reported.
Duan (1959) cited in AB Health. Rats exposed to 10 ppm H_2S for 12 hrs/day, 5 days/wk for 90 days	Slight irritation of the mucous membranes of the trachea and bronchi were reported.
Fyn-Zhuy (1959) Rats were exposed to 0.2 or 10mg/m3 H_2S daily for 3 months	Concentrations of 10mg/m3 caused irritation of the mucous lining of the trachea and bronchi, where as the 0.2mg/m3 concentrations the irritation was less pronounced.
Heymans et al. (1931)	Two series of experiments with dogs show that the stimulating action of Na_2S on respiration is largely reflex from the carotid sinus and is only very slightly due to a direct stimulating effect on the respiratory center.

Table A26 Respiratory System Effects: *In vitro* Studies

Reference / Study Design	Key Findings
Husain (1976) and Husain and Zaidi (1977) Rat lung tissue homogenate was exposed to 18, 46 and 108 ppm H_2S for 1 hour in the cold. Its effect on lung enzyme activity was measured.	At 18 ppm, the following enzymes were inhibited: acid phosphatase (16.8%), alkaline phosphatase (11%), glutamic pyruvate transaminase (GPT) (25.9%), glutamic oxaloacetic transaminase (GOT) (15.9%), and adenosine triphosphatase (ATPase) (13.3%). As the H_2S concentration increased, the enzymes were inhibited in a dose-response manner. Arginase activity increased with all gas exposures and aldolase activity did not change with any exposure.
Mirza (1976) The effect of various concentrations of H_2S gas on the activities of rat lung enzymes was tested in vitro using a simple gas bubbling method.	ATPase, acid phosphatase, alkaline phosphatase, glutamic pyruvic transaminase and glutamic-oxaloacetic transaminase activities were inhibited. Arginase activities were increased with various concentrations. Fructose-1,6-diphosphate aldolase activity was unaffected.
Pashchenko (1969) The effects of acute, sub-acute and chronic exposures to H_2S and methyl mercaptan (CH3SH) on ATPase in brain, skeletal muscles, kidney, lung, heart and spleen in mice were studied. Acute exposures were to 570 - 714 ppm H_2S and 2000 - 2500 ppm CH3SH; sub-acute exposures were carried out 3 hr/day for 3 weeks at 72 - 144 ppm H_2S and 500 - 1000 ppm CH3SH; and chronic exposures were daily for 3 hrs during 3 months at 29 - 43 ppm H_2S and 200-300 ppm CH3SH.	Acute H_2S poisoning decreased ATPase activity 26% in the lungs, and subacute H_2S poisoning decreased brain ATPase 14%. Acute CH3SH exposure significantly decreased activity in the brain, lungs, and spleen and increased activities were found in the brain and spleen after chronic CH3SH poisoning.
Jonek and Knoecki (1966) Rabbits were exposed to 71 ppm H_2S for 30 minutes daily for 7, 10, and 14 days. The animals were sacrificed and their lungs excised and sectioned.	A decrease in acid phosphatase, alkaline phosphatase, adenosine triphosphatase, and deoxyribonuclease was observed.

APPENDIX II

Synopsis of Selected Anecdotal Reports

I. Round (1992)

In cattle and swine, especially calves and piglets, clinical signs of upper respiratory problems and eye irritation predominated (signs included rhinitis, slight serous discharge from the nose, sneezing (piglets) and coughing (calves), conjunctivitis). There were normal body temperatures and virtually no response to medications.

Over a ten-year interval a cow-calf operator had an essentially non-existent incidence of respiratory diseases; during the blowout, his calves weaned two months previously had signs of upper respiratory tract disease, which following the capping of the well, disappeared. The signs have not reappeared since the blowout.

Clinical signs of eye irritation (reddening of the conjunctiva and sclera, congestion of the eye and tearing) were observed during the blowout and also when Dome West Pembina started up their gas plant; these signs were always accompanied with signs of upper respiratory irritation.

Aborted cattle fetuses were sent to the veterinary diagnostic laboratory and after extensive procedures, no known cause of abortion could be diagnosed. The Lodgepole blowout occurred concurrently with the first cold weather of the season, which, by itself, is associated with an increase in abortions, thus confounding the identification of the effects of the blowout. However, the chemicals emitted must be suspect, especially carbon disulfide. One animal, which was born just after the blowout was capped, from the veterinarian's own herd, failed to have normal estrus cycles. Ovulation was induced and following conception, pregnancy was maintained until the seventh month, when she aborted.

Nursing sows were affected with clinical signs of eye and upper respiratory irritation, and aggression. The aggressive sows were poor mothers and would trample and crush the piglets. They would not relax while nursing; rather they would bark, get up, step around and then lie down and crush or injure piglets. In addition to stillbirths, weaning weights of the surviving piglets were decreased by about 33%.

Some farmers averaging 10 - 12 piglets/sow before the blowout would get 3 live piglets and 7 - 9 dead. Sows bred and farrowed subsequent to the blowout produced 10 - 12 piglets.

The veterinarian had a gilt, in gestation during the blowout that developed lymphosarcoma.

During plant upsets, prior to the blowout, the veterinarian had 100 feeder pigs that were doing quite well. When the flaring occurred, the pigs developed 'red eye'.

II. Church (1992)

Beef cattle producers complained about open cows, increased number of abortions, and increased numbers of late calves.

Four producers complained of poor growth and milk production in replacement heifers which had been exposed to the blowout at an early age; two reported abnormal breeding cycles, and abnormal hair color in some herds; most of these problems were prevalent in 1983, although the number of late calves increased from 1983 - 1985.

An increased percentage of beef calves borne after the blowout were small, weak and non-aggressive.

There was also a higher number of stillborn and defective calves born in 1983; two common problems reported were deformed front legs and light weaning weights (60 - 100 lbs lighter than average).

Scours was also identified as a problem on some farms.

One dairy farmer experienced a 20% decrease in milk production for three months; calving interval decreased from 13.9 months in 1983 to 11.8 months in 1985.
A swine producer recorded an increase in abortions and deformities from 1982 to 1983. Some brood sows were culled and replacement sows, which had been exposed to sour gas, demonstrated abnormal cycling, poor performance, and small litters. The number of pigs/sow/year decreased from 16.8 in 1980 and 20 in 1981, to an average of 12 for 1982 - 1984.

In a purebred Angus herd exposed to emissions from the blowout, owners identified 8 problems: average decrease of 15 pounds birthweight; 4.6% increase in birth defects of stillborn calves; 100 pound decrease in weaning weight; hair color faded and greying; 23% increase in culling rate; 9.5% increase in abortions; decreased growth and milking ability of heifers born in 1983; and breeding problems such as abnormal cycling.

III. Ludwig (1997)

The Ludwig's describe their experiences with oil and gas industry encounters from 1990 - 1997. Three sour gas leaks affecting the Ludwig family were highlighted. The first was a release of 60 m^3 of sour gas from a well 0.5 miles away from the Ludwig's residence on Jan 23, 1991. Miscarriage affecting 50% of their lamb and goat kids was reported following the leak. "A raft of unusual human illness" (specifics were not given) was also reported. It was noticed that a rabbit's gall bladder was three times larger than it should be; this had never been seen before. The second release was a release of 1694m^3 sour gas over a 45-minute period on Dec 12, 1993, which required evacuation of the family from their home. The Ludwig's experienced nausea, headache, and vomiting. They were 'sick and scared'. After leaving the area, the previously chronic skin problems cleared up. The third leak, on Aug 30, 1996, was from a well to the southeast of the farm. Nausea and headaches were experienced within minutes of the leak occurring.

Several other people living in the same vicinity also described their experiences living near oil and gas operations. A farmer from Sexsmith also had to leave his residence when, under heavy rain and a strong east wind occasionally extinguished a burning flare. The farmer described it as an awful smell causing eyes to tear, choking and an upset stomach.

A women who lived near Hythe, had raised goats for 25 years. In 25-yr period she had never lost a kid or ever had a deformed kid. After the AEC Hythe gas plant was built, in the winter of 93 - 94, from 7 - 9 does, 18 kids were born and none of them lived. Some were full term but died a couple of days later; some were spontaneous abortions.

Another farmer noticed that after a Norcen gas well was drilled near his house, people had stomach problems shortly after. After switching to bottled water, the stomach problems disappeared. There was combustible gas dissolved in the water, enough to ignite directly from the tap.

IV. Kostuch (1999) presented observational evidence of the impact of air pollution on animal health.

- Reproductive effects seen include: increased incidence of infections that results in poor uterine tone and reduced fertility; increased uterine inertia (the cows don't push during calving); poor heat signs; prolonged calving intervals, increase in abortions and stillbirths.
- Respiratory signs are more pronounced with acute exposure: runny eyes, runny noses, 'gas' eyes, and burned back ends (resembling urine scald); coughing, and increased incidents of pneumonia.
- Immuno-suppression effects: there is increased susceptibility and less resistance to disease, leading to increased incidence of foot-rot, pinkeye, pneumonia, and other diseases.
- Increased deficiencies arise from excess sulphur; sulphur interacts antagonistically with copper, selenium and zinc. Farmers are feeding 5 - 10 times the recommended levels in an effort to overcome the deficiencies induced by sulphur antagonism; deficiencies of selenium, copper and zinc lead to weak, unthrifty calves, increased incidence of white muscle disease, stupid calves, calves with difficulty sucking, lameness, difficulty breathing, sudden death, downed cows, chronic diarrhea, and hoof problems.
- Neurological problems may be related to some of the complex compounds and include staggering, poor mothering and aggressive behavior.

V. Johnston (1999)

High H_2S readings in the previous three days were thought to be related to strange behavior of animals, calves reluctant to rise when disturbed, cows having difficulty walking, and 30 - 40 yearlings had extremely bad eyes.

In the space of one week (late April 1997) a series of flaring incidents occurred (Tues., Thurs., Fri., Sat., Sun morning and afternoon, Mon.) which were followed by severe lung, nasal and eye irritation, and dead animals.

Three days after the flaring of a new sour gas well 3 miles away, a dead 6-week old calf that previously had shown no signs of illness was found.

VI. Whitelock (1999)

During flare testing cattle not pushing or straining while calving.

Calves borne with 'non-existent' immune systems; walking on their ankles or not walking; scours and pneumonia corresponding to a peak H_2S measurement of 5.9 ppm.

VII. Bocock (1999)

Abnormal sexual behavior in cows, bulls, heifers, tomcats, wild ducks and crows located near a sour gas processing site

11/45 calves born healthy with healthy dam in fall 1996.

3 sets of twins born out of 45 births (2 sets/100 births considered normal); one set began to rot before birth; one cow expelled rotted fetus about 23 days after expected delivery time.

More common effects were cows with no milk until a day or more after calving; calves with impaired vision, hyperactive calves, and also 'dumb calf' syndrome.

Respiratory problems, including emphysema and pneumonia; younger calves were always more affected with these problems.

5/23 heifers on a pasture affected by an oil battery developed lesions that rendered them infertile.

3/23 heifers on the same pasture developed bull-like traits and behavior and had to be slaughtered.

5/9 heifers in another season aborted and had to be culled.

23 sets of twins (from a herd smaller than after) during time oil battery operated; 2 sets of twins after.

VIII. 2000 - Sour Gas Public Safety Review

In June 2000, 223 individuals, some of who were there on behalf of larger groups or organizations, attended twelve open houses. Air quality, public and animal health concerns were raised at all of the meetings. In order of frequency, the areas identified include: respiratory problems 5x (sore throats 2x, asthma 4x, cough); negative mental health 5x (stress, anxiety, frustration, worry); cancer 5x; headache 4x; nausea 4x; reproductive problems 3x (premature births); allergies 2x, runny eyes 2x, sore eyes 2x, nosebleeds, autoimmune disorders 2x, feeling intoxicated, strokes, heart attacks, insomnia, brain damage, growth problems, death.

APPENDIX III

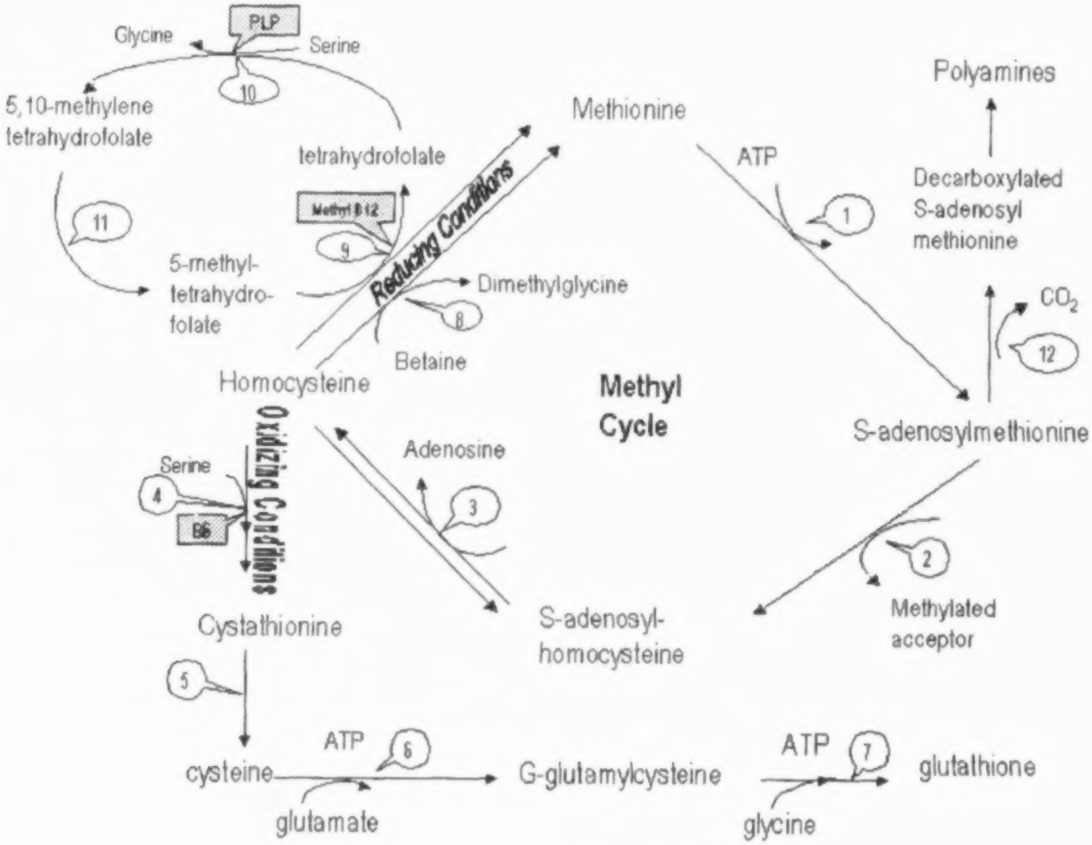

Figure A1 The Methyl Cycle

B6 – Vitamin B6; B12 – Vitamin B12; PLP – Pyridoxal Phosphate; (1) Methionine adenosyltransferase; (2) transmethylation reactions; (3) S-adenosylhomocysteine hydrolase; (4) cystathionine β-synthase; (5) γ cystathionase; (6) γ-glutamylcysteine synthetase; (7) glutathione synthetase; (8) methionine synthetase; (9) betaine homocysteine methyltransferase; (10) serine hydroxymethylase; (11) methylenetetrathydrofolate reductase; (12) S-adenosylmethionine decarboxylase (Adapted from Finkelstein, 1998; Taoka et al., 1996)